final Counts of 87N972 plaque assays (Cumulative)

Ad5 1	106	1041 19	88	801 37	120	DME 55	0
2	83	20	108	38	114	56	0
3	84	21	95	39	123	57	(all cells dead)
4	13	22	14	40	16		
5	12	23	14	41	12		
6	5	24	12	42	12		
1035 7	omit → cells dead	918 25	78	716 43	1		
8	69	26	74	44	1		
9	68	27	71	45	1		
10	11	28	14	46	0		
11	9	29	8	47	1		
12	9	30	10	48	0		
1038 13	110	305 31	41	712 49	46		
14	107	32	37	50	57		
15	85 (but high cell death)	33	41	51	52		
16	16	34	5	52	– (total cell death)		
17	7	35	1	53	6		
18	9	36	6	54	8		

Ad5 → distinct plqs
1035 → not as definitive
1038 →

1041 → more distinct plq
918 → distinct
305 → distinct

large plaques
716 → largest, most distinct plaques

Small plaques
801 → very small & slow growth

712 → very small with slow growth

FROM :SA[illegible] EXHIBIT A2 [illegible]MI FAX NO. :3147733403 Dec. 19 2002 10:23AM P2

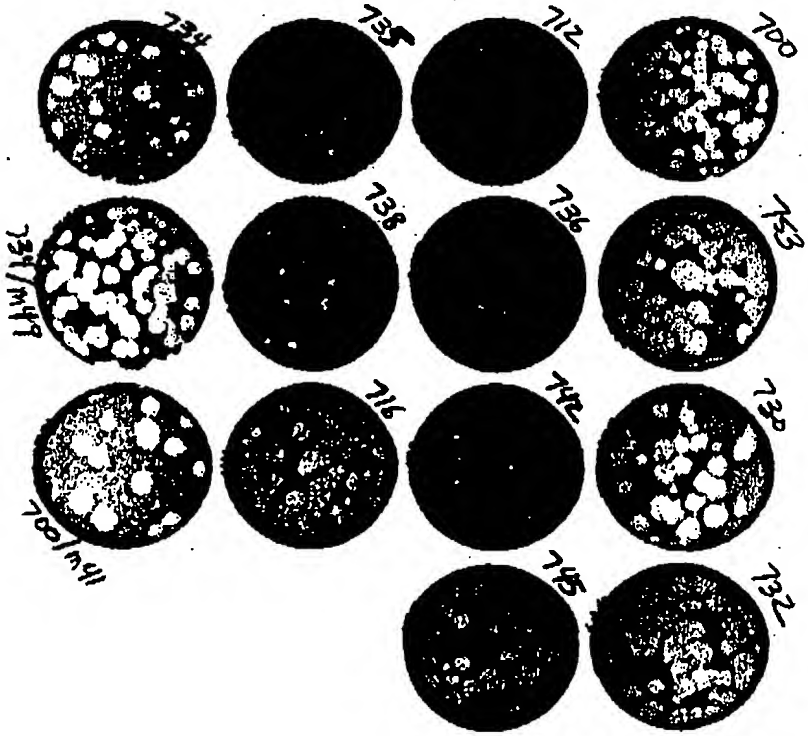
11.6K → possibly related to plaque size + morphology?

(tend to vary more in plaque size)

Plaque Size

Large + distinct	Smaller less distinct	Small
dl 753 (esp.)	most 5PY 10.4K mutants	748
dl 754		712
dl 714	[pm 775 more distinct + larger]	dl 801
dl 778	pm 771	dl 766
Ad 5 (esp.)	dl 713	dl 742
dl 799	dl 327 (smaller than rec700 in dl 728)	pm 785
~~[illegible]~~		In 724
dl 716 (esp.)		dl 748
dl 751 (esp.)		dl 706
dl 718		dl 708
		dl 717
		dl 7001

E3-11.6k Small Plaque Mutants



Early work
on defining
ADP function

EXHIBIT
A3

EXHIBIT
A4

Plaque Assay — (11.6K Mutants → plaque assay; to test)

11 P.A. dishes

Virus	Prep	Titer	$5*10^{-9}$	$.5*10^{-10}$
①	rec700 (VS188)(910513)	$2.37*10^{11}$	2	3
②	dl742 (860313)	$1.77*10^{11}$	2	3
③	dl745 (860313)	$2.06*10^{11}$	2	3
④	dl712 (plq1)(VS204)(911101)	$1.56*10^{11}$	2	3
⑤	dl730 (39-4)(VS168)(901214)	$1.73*10^{11}$	2	3
⑥	dl732 (2%)(AS)(890414)	$3.4*10^{11}$	2	3
⑦	pm734 (1%)(890504)	$6.7*10^{10}$	3	2
⑧	dl735 (3%)(890425)	$9.*10^{10}$	3	2
⑨	dl736 (0.01%)(890414)	$2.88*10^{10}$	3	2
⑩	dl738 (38-11)(VS171)(910118)	?	3	3
⑪	700/M41 (AS)(920214)	?	3	3
⑫	pm734/M49 (AS)(920402)	?	3	3
⑬	dl716 (plq. 4)(VS125)(900202)	$2.04*10^{11}$	1	2
⑭	dl753 (VS78)(880624)	$1.42*10^{11}$	1	2
⑮	mock	—	1 (5/1)	

5F-DME for all pre-infection;
infection with 0.5 ml of dilution at 4:30pm;
added 6 ml of overlay at 5:45pm;

(5/29) added 5 ml of NR overlay; one dish of
dl 738 ($.5*10^{-9}$ dilution) dropped → discarded

on (5/29) see "large" distinct plaques on dl 730, dl732,
dl 716, dl 753 dishes (+ pair dl 745), rec 700 may
have a few smaller plaques & really do not
see plaques on other dishes yet.

EXHIBIT A5

(5/21) plaques marked in red
(6/3) marked in green

Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
① rec700 (VS188)(910503)	.5*10^-9	+39 55	+35 51	—	quite large + distinct; vary in size & how distinct (smaller & less distinct than 730, 732, 753)
	.5*10^-10	+8 2	+4 2	+4 3	
② dl742 (860313)	.5*10^-9	+2 3	+5 2	—	very indistinct
	.5*10^-10	0 0	+1 0	0 0	very tiny + indistinct
③ dl745 (860313)	.5*10^-9	+20 24	+13 22	—	quite large to slightly larger; quite distinct; similar size to rec700
	.5*10^-10	+2 5	+1 1	+2 3	
④ dl712 (plg 1) (VS204)(911101)	.5*10^-9	+8 0	+6 0	—	no plqs. visible (at least not definitive); very small plaques
	.5*10^-10	+1 0	0 0	0 0	
⑤ dl730 (39-4) (VS168)(901214)	.5*10^-9	+16 20	+22 20	—	quite large & distinct; probably larger than rec700
	.5*10^-10	+1 1	+4 3	+1 7	
⑥ dl732 (29/1) (A5)(890114)	.5*10^-9	+8 72	+12 52	—	larger & more distinct than dl730 & dl753; large & very distinct
	.5*10^-10	+3 4	+3 1	+3 5	
⑦ pm734 (1/2) (870504)	.5*10^-9	+16 4	+8 1	+20 5	probably not very small, but very indistinct; prob. smaller than rec700
	.5*10^-10	+4 0	+2 0	—	
⑧ dl735 (34/1) (890425)	.5*10^-9	+8 6	+1 0	(...) +1 3	indistinct plaques, size not very definite
	.5*10^-10	0 0	+1 0	—	
⑨ dl736 (0.0/1) (870414)	.5*10^-9	+1 1	+2 1	+4 1	very small + indistinct; indistinct plaques
	.5*10^-10	0 0	0 0	—	very small + indistinct

EXHIBIT
A6

(>31) plaques
(6/3) marked in green

Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (VS171)(910118)	.5*10^-9	+6 4	+1 11	—	not too small, but not very distinct
	.5*10^-10	0 0	0 0	0 0	small to indistinct
(11) 700/m41 (AS)(920214)	.5*10^-9	+7 8	+3 11	+12 10	~ like rec 700 quite large & very distinct
	.5*10^-10	+0 2	+2 0	+1 2	
(12) pm 734/m47 (AS) (920402)	.5*10^-9	+55 28	+13 31	+46 36	~ like rec 700
	.5*10^-10	+11 3	+6 4	+7 1	~ like rec 700
(13) dl 716 (plq 4) (VS125)(900202)	.5*10^-9	+26 62	—	—	quite large & distinct
	.5*10^-10	+1 5	+1 3	—	larger & more distinct than rec 700
(14) dl 753 (VS78)(880624)	.5*10^-9	+15 42	—	—	quite large & distinct
	.5*10^-10	+2 2	+0 3	—	very large & distinct
(15) mock	—	0 0	—	—	good monolayer

Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
① rec 700 (VS 188)(910503)	$.5*10^{-9}$	+13	+16	—	plaques quite large + distinct, new plaques distinct
	$.5*10^{-10}$	+0	+3	+1	
② dl 712 (860313)	$.5*10^{-9}$	+6	+5	—	new plaques very indistinct, older plaques still small with diffuse edges
	$.5*10^{-10}$	0	+1	0	
③ dl 745 (860313)	$.5*10^{-9}$	+7	+7	—	large, distinct plaques; new plaques very distinct
	$.5*10^{-10}$	+2	+0	+1	
④ dl 712 (plq 1) (VS 204)(911101)	$.5*10^{-9}$	+6	+10	—	small + indistinct
	$.5*10^{-10}$	+0	0	0	older plqs still small
⑤ dl 730 (39-1) (VS 168)(901214)	$.5*10^{-9}$	+6	+8	—	very large + very distinct plaque new plqs distinct
	$.5*10^{-10}$	+1	+1	+2	
⑥ dl 732 (27/1) (AS)(890414)	$.5*10^{-9}$	+4	+2	—	very large + distinct plaque
	$.5*10^{-10}$	+0	+4	+1	
⑦ pm 734 (19/2) (890504)	$.5*10^{-9}$	+19	+17	+13	plaques a bit smaller than rec 700; new plaqs quite distinct
	$.5*10^{-10}$	+2	+1	—	
⑧ dl 735 (34/1) (890425)	$.5*10^{-9}$	+6	+12	+13	small + very indistinct plaq
	$.5*10^{-10}$	+1	+1	—	
⑨ dl 736 (0.04/1) (890414)	$.5*10^{-9}$	+5	+5	+6	very small + very indistinct (both old + new)
	$.5*10^{-10}$	+1	0	—	

(9/5) [illegible]

EXHIBIT A8

Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (VS171)(910118)	$.5*10^{-9}$ $.5*10^{-10}$	+5 +1	+6 0	— 0	new plaques very small & indistinct; even older plaques are ~ same size with diffuse edges
(11) 700/m41 (AS)(920214)	$.5*10^{-9}$ $.5*10^{-10}$	+5 +0	+2 +0	+5 +0	plaques large, new plaques very distinct (similar size to much older dl738)
(12) pm 734/m49 (AS) (920402)	$.5*10^{-9}$ $.5*10^{-10}$	+17 +4	+16 +1	+17 +5	new plqs very distinct! fairly large & distinct, maybe a bit smaller than rec700 plaques
(13) dl 716 (plq 4) (VS125)(900202)	$.5*10^{-9}$ $.5*10^{-10}$	+1 +1	— +1	— —	very large to distinct ([illegible] on 10^{-11})
(14) dl 753 (VS 78)(880624)	$.5*10^{-9}$ $.5*10^{-10}$	+7 +1	— +2	— —	very large & distinct
(15) mock	—	0	—	—	good monolayer

EXHIBIT
A9

Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
(1) rcc 700	.5*10^{-9}	+3	+6	–	distinct fairly
(vs 138)(910503)	.5*10^{-10}	+1	+1	+1	large plaques
(2) dl 742 (860313)	.5*10^{-9}	+20	+22	–	small & indistinct plaques; new
	.5*10^{-10}	+3	+2	+1	plaques "pinpoint"
(3) dl 745 (860313)	.5*10^{-9}	+7	+2	–	large distinct
	.5*10^{-10}	+0	+0	+1	plaques
(4) dl 712 (ply 1)	.5*10^{-9}	+28	+29	–	very small plqs;
(vs 204)(911101)	.5*10^{-10}	+1	+1	+2	new plqs "pinpoint"
(5) dl 730 (39-4)	.5*10^{-9}	+1	+4	–	very large, nice
(vs 168)(901214)	.5*10^{-10}	+1 (mold)	+0	+0	plaques (plqs a bit diffuse)
(6) dl 732 (29/1)	.5*10^{-9}	+7	+1	–	very large, nice
(AS)(890414)	.5*10^{-10}	+0	+0 (mold)	+1	plaques
(7) pm 734 (11/2)	.5*10^{-9}	+28	+28	+25	slightly smaller plaques; new
(890504)	.5*10^{-10}	+2	+3	–	plaques fairly lg. & distinct
(8) dl 735 (3/1)	.5*10^{-9}	+18	+34	+30	older plqs tiny
(890425)	.5*10^{-10}	+5	+7	–	new plqs – "pinpoints"
(9) dl 736 (0.04/1)	.5*10^{-9}	+11	+22	+15	very small plqs
(890414)	.5*10^{-10}	+0	+0	–	new plqs "pinpoints"

(10) marked in black

EXHIBIT A10

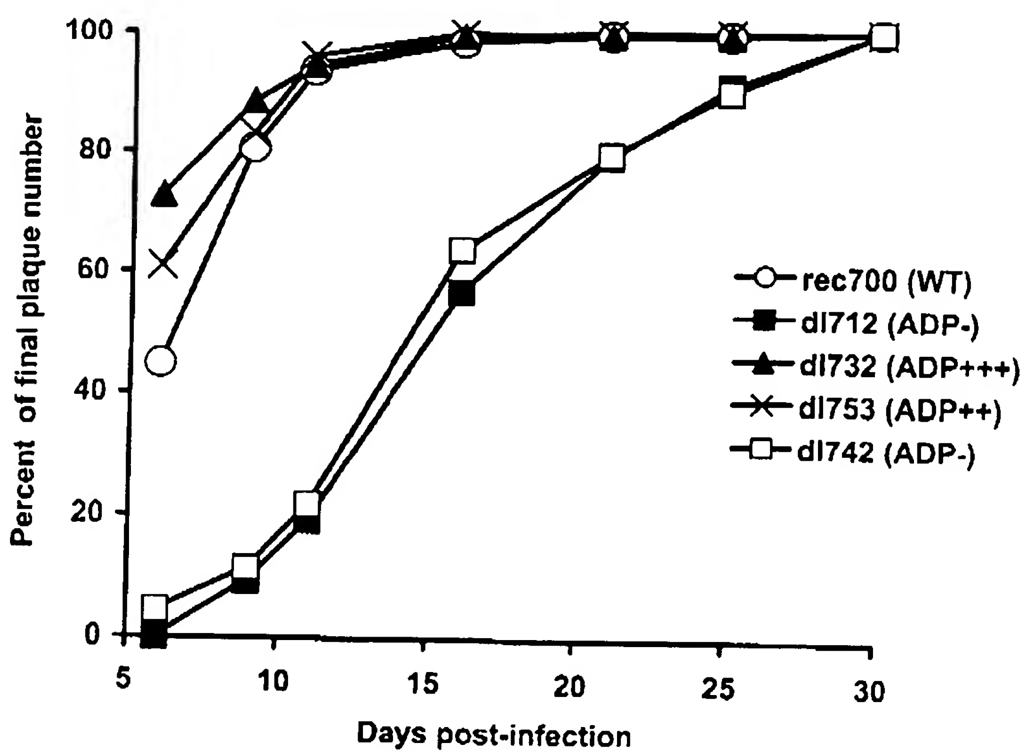
Counts of (5/25) plaque Assays

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (VS171)(910118)	.5*10^-9 .5*10^-10	+22 +2	+16 +3	— +3	small + indistinct, near plaques very small
(11) 700/m41 (AS)(920214)	.5*10^-9 .5*10^-10	+3 +1	+5 +0	+4 +0	new plqs larger than those of dl 738; large plaques + distinct
(12) pm 734/m41(AS) (920402)	.5*10^-9 .5*10^-10	+5 +2	+7 +3	+15 +4	large distinct plaques (new plqs larger than oldest (738)
(13) dl 716 (plq 4) (VS125)(900202)	.5*10^-9 .5*10^-10	+3 +1	— +1	— —	very large + distinct
(14) dl 753 (VS 78)(880624)	.5*10^-9 .5*10^-10	+1 +0	— +2	— —	very large + distinct
(15) mock	—	0	—	—	good monolayer

EXHIBIT

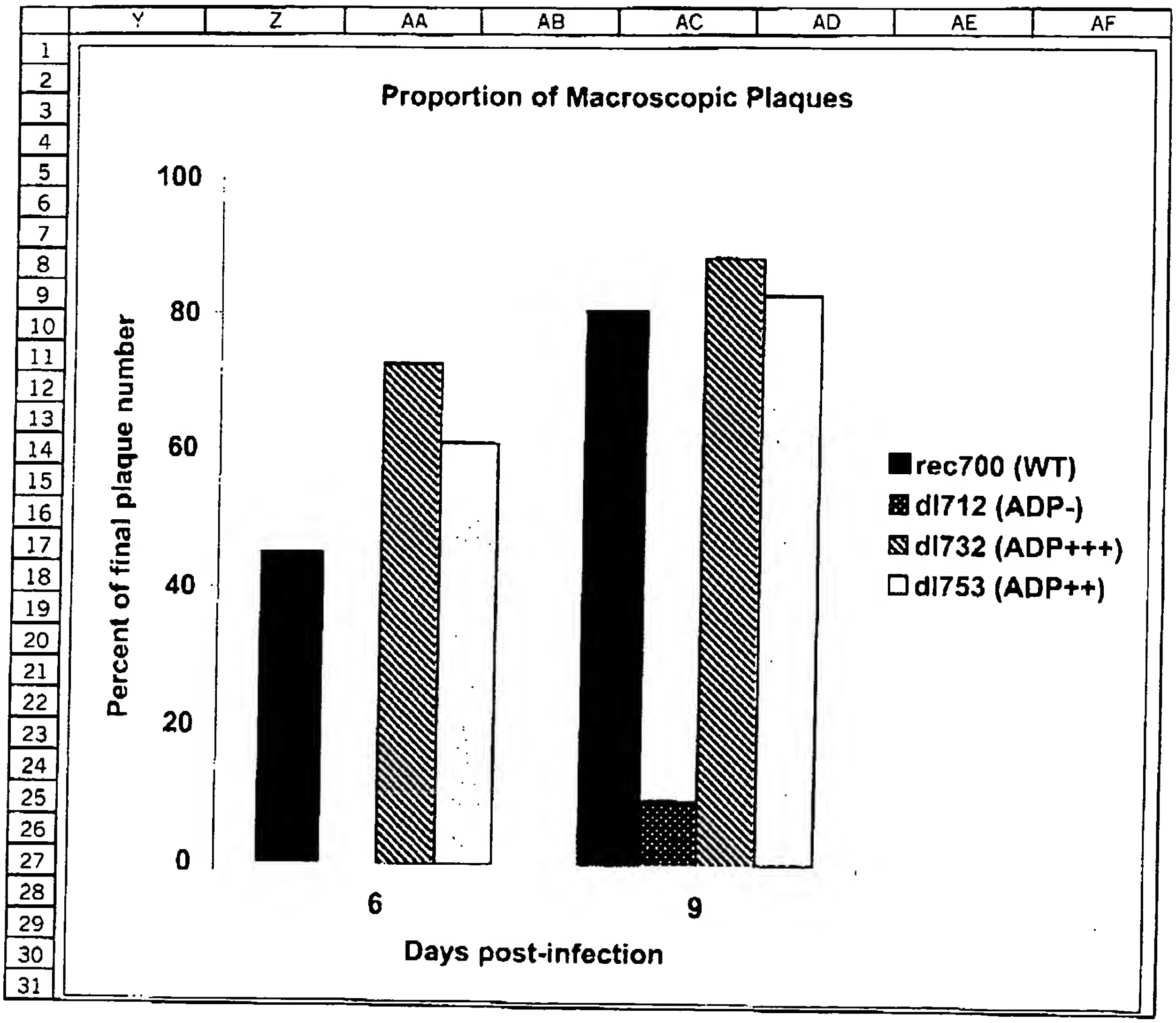
A11

plaque assay



EXHIBIT

A12



Y
Z
AA
AB
AC
AD
AE
AF
Proportion of Macroscopic Plaques
100
80
60
40
20
0
Percent of final plaque number
6
9
Days post-infection
rec700 (WT)
dl712 (ADP-)
dl732 (ADP+++)
dl753 (ADP++)

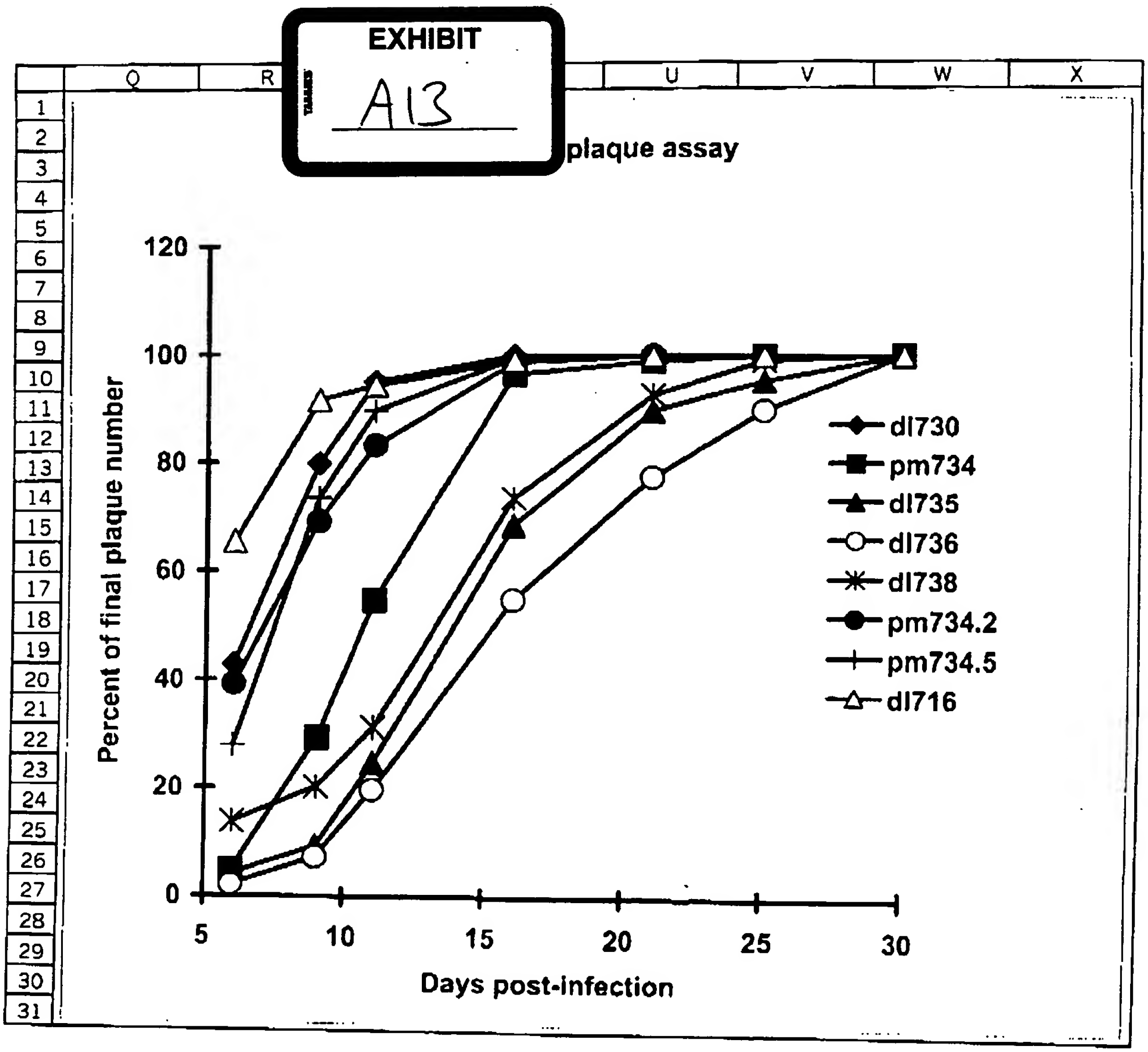


EXHIBIT
A13
plaque assay
Q
R
U
V
W
X
Percent of final plaque number
120
100
80
60
40
20
0
5
10
15
20
25
30
Days post-infection
dl730
pm734
dl735
dl736
dl738
pm734.2
pm734.5
dl716

EXHIBIT
A14

	A	B	C	D	E	F	G	H
2		6	9	11	16	21	25	30
3	rec700 (WT)	44.8	80.6	93.7	98.4	99.6	99.6	100
4	dl712 (ADP-)	0	9.3	19.3	57.1	79.5	91.3	100
5	dl732 (ADP+++)	72.8	88.6	94.6	99.5	99.5	99.5	100
6	dl753 (ADP++)	61	83.1	96.1	100	100	100	100
7	dl742	4.4	11.4	21.9	64	79.8	90.4	100
8	dl745	44.7	75.6	89.4	97.6	99.2	99.2	100
9		6	9	11	16	21	25	30
10	dl730	42.9	79.8	95.1	100	100	100	100
11	pm734	4.9	29.3	54.6	96.6	99	100	100
12	dl735	4.2	9.3	24.7	68.4	89.8	95.3	100
13	dl736	2.2	7.3	19.7	54.7	77.4	89.8	100
14	dl738	13.8	20.2	31.2	73.4	92.7	99.1	100
15	pm734.2	39.3	69	83.3	98.8	100	100	100
16	pm734.5	27.9	73.4	89.7	99.5	100	100	100
17	dl716	65.4	91.6	94.4	99.1	100	100	100
18								
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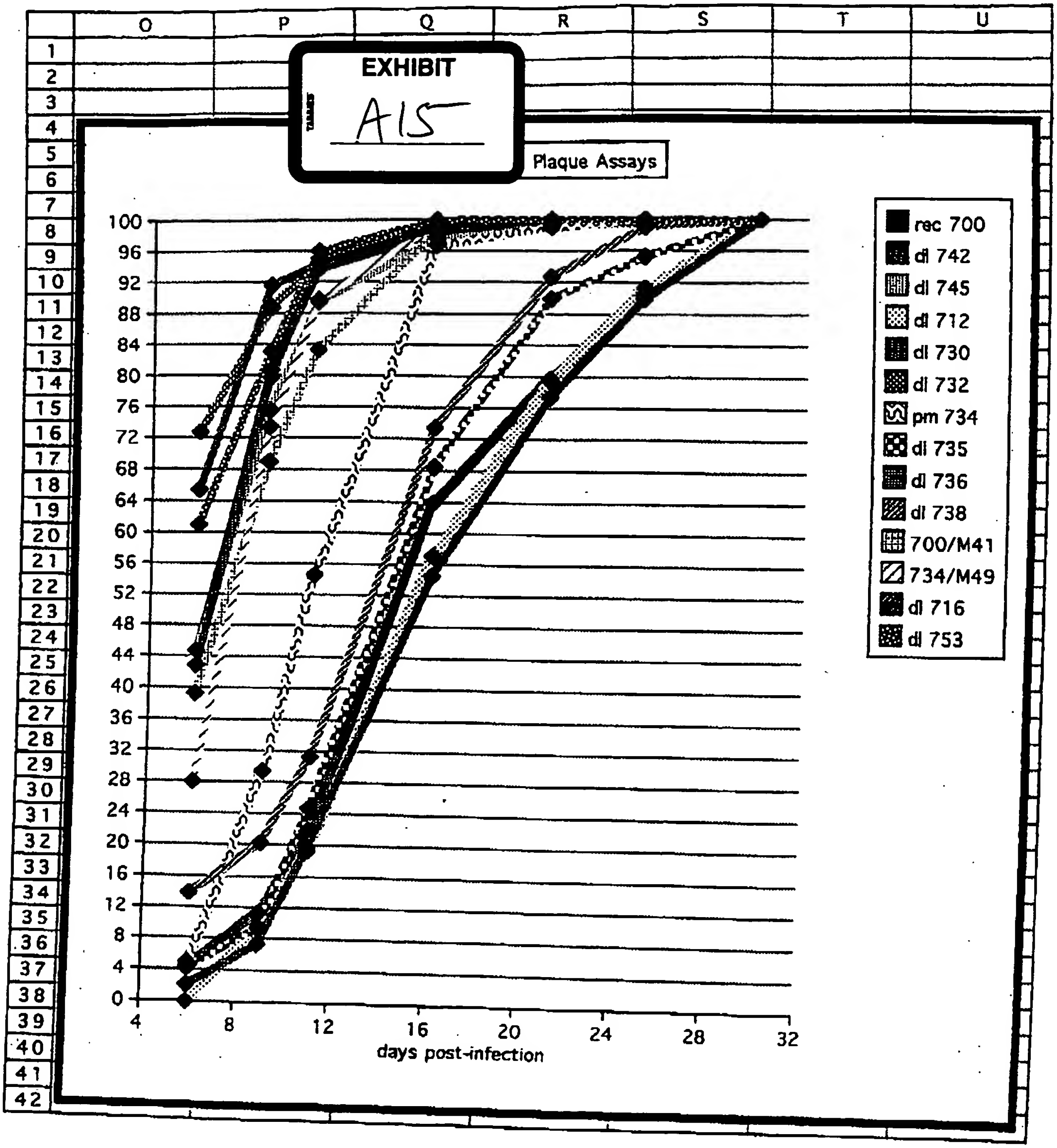
31								

EXHIBIT A15



Plaque Assays
100
96
92
88
84
80
76
72
68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
4
8
12
16
20
24
28
32
days post-infection
rec 700
dl 742
dl 745
dl 712
dl 730
dl 732
pm 734
dl 735
dl 736
dl 738
700/M41
734/M49
dl 716
dl 753

EXHIBIT
A16

ac 700	6	9	11	16	21	25
	44.8	80.6	93.7	98.4	99.6	99.6
dl 742	6	9	11	16	21	25
	4.4	11.4	21.9	64	79.8	90.4
dl 745	6	9	11	16	21	25
	44.7	75.6	89.4	97.6	99.2	99.2
dl 712	6	9	11	16	21	25
	0	9.3	19.3	57.1	79.5	91.3
dl 730	6	9	11	16	21	25
	42.9	79.8	95	100	100	100
dl 732	6	9	11	16	21	25
	72.8	88.6	94.6	99.5	99.5	99.5
pm 734	6	9	11	16	21	25
	4.9	29.3	54.6	96.6	99	100
dl 735	6	9	11	16	21	25
	4.2	9.3	24.7	68.4	89.8	95.3
dl 736	6	9	11	16	21	25
	2.2	7.3	19.7	54.7	77.4	89.8
dl 738	6	9	11	16	21	25
	13.8	20.2	31.2	73.4	92.7	99.1
700/M41	6	9	11	16	21	25
	39.3	69	83.3	98.8	100	100
734/M49	6	9	11	16	21	25
	27.9	73.4	89.7	99.5	100	100
dl 716	6	9	11	16	21	25
	65.4	91.6	94.4	99.1	100	100
dl 753	6	9	11	16	21	25
	61	83.1	96.1	100	100	100

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :314 . 19 2002 10:30AM P16

EXHIBIT
A17

Y.A. (p.2)

30						
100						
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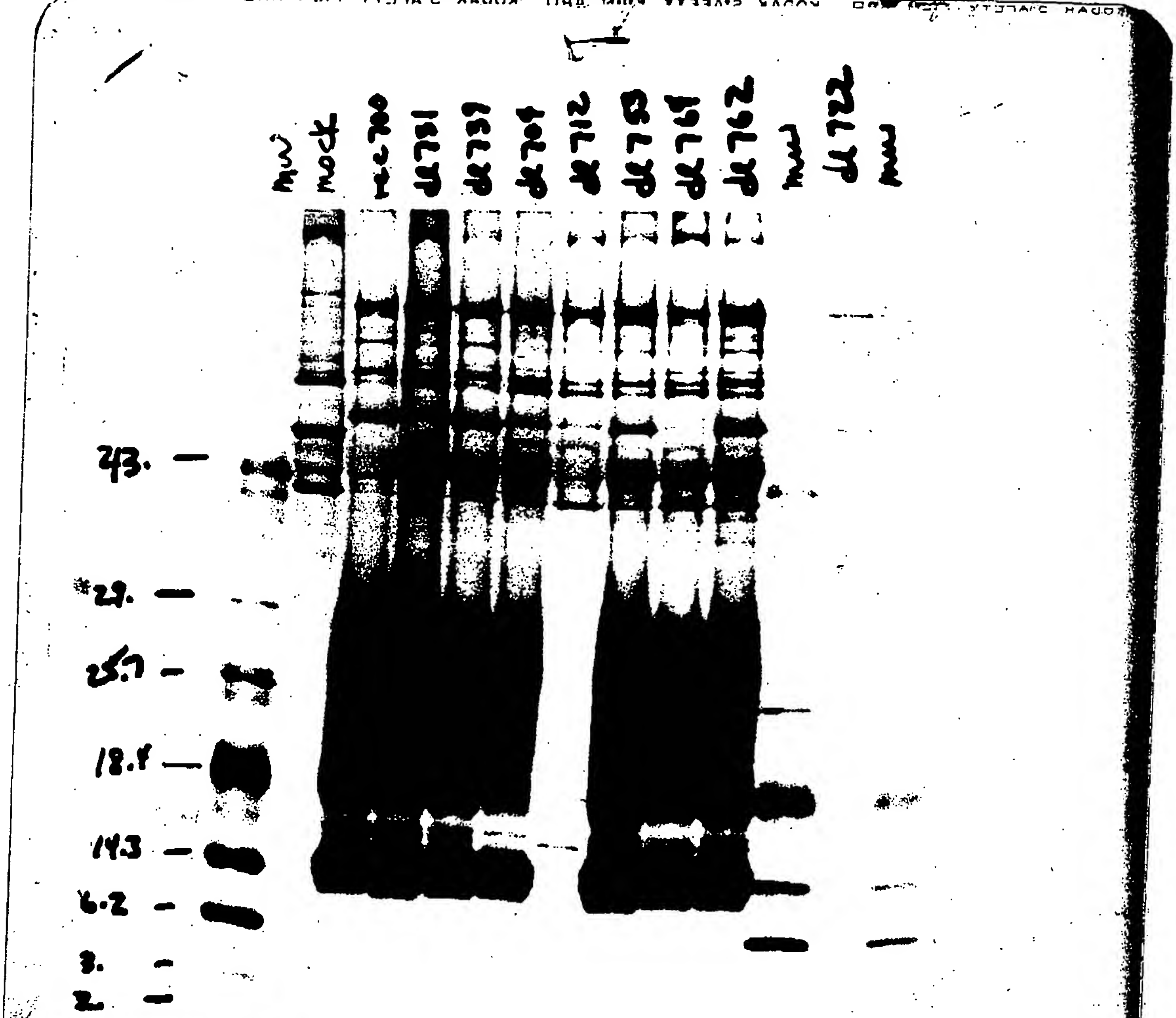


EXHIBIT
A18

Gel #1
13 day exposure
5 min developer 0709 #1

11.6 (Deletion Mutants)

8/9 →

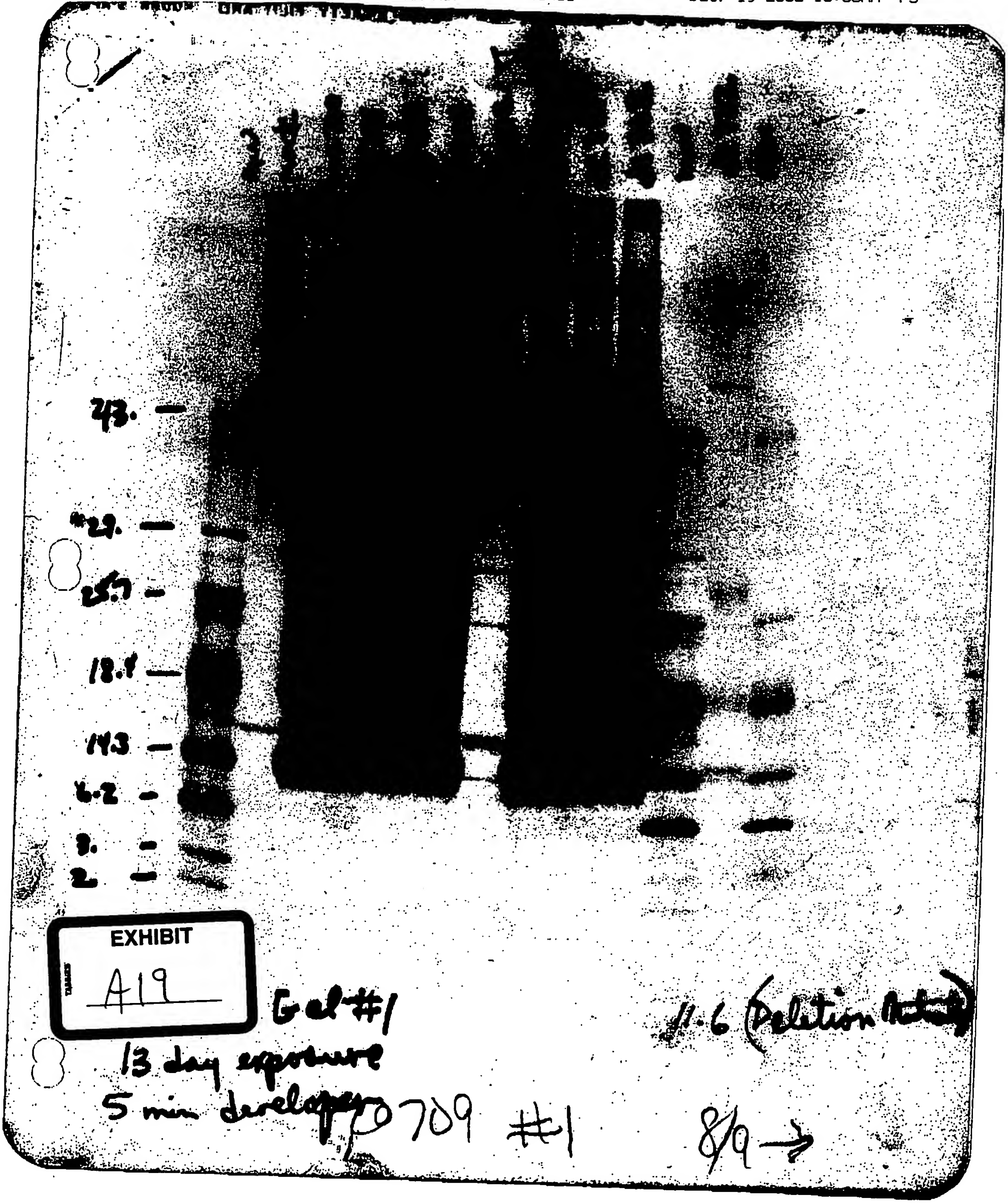

43.
29.
25.7
18.4
14.3
6.2
3.
2.
EXHIBIT
A19
Gel #1
13 day exposure
5 min developer
11.6 (Deletion
D709 #1
8/9 →

EXHIBIT
A20

7 day exposure (5 min developer)

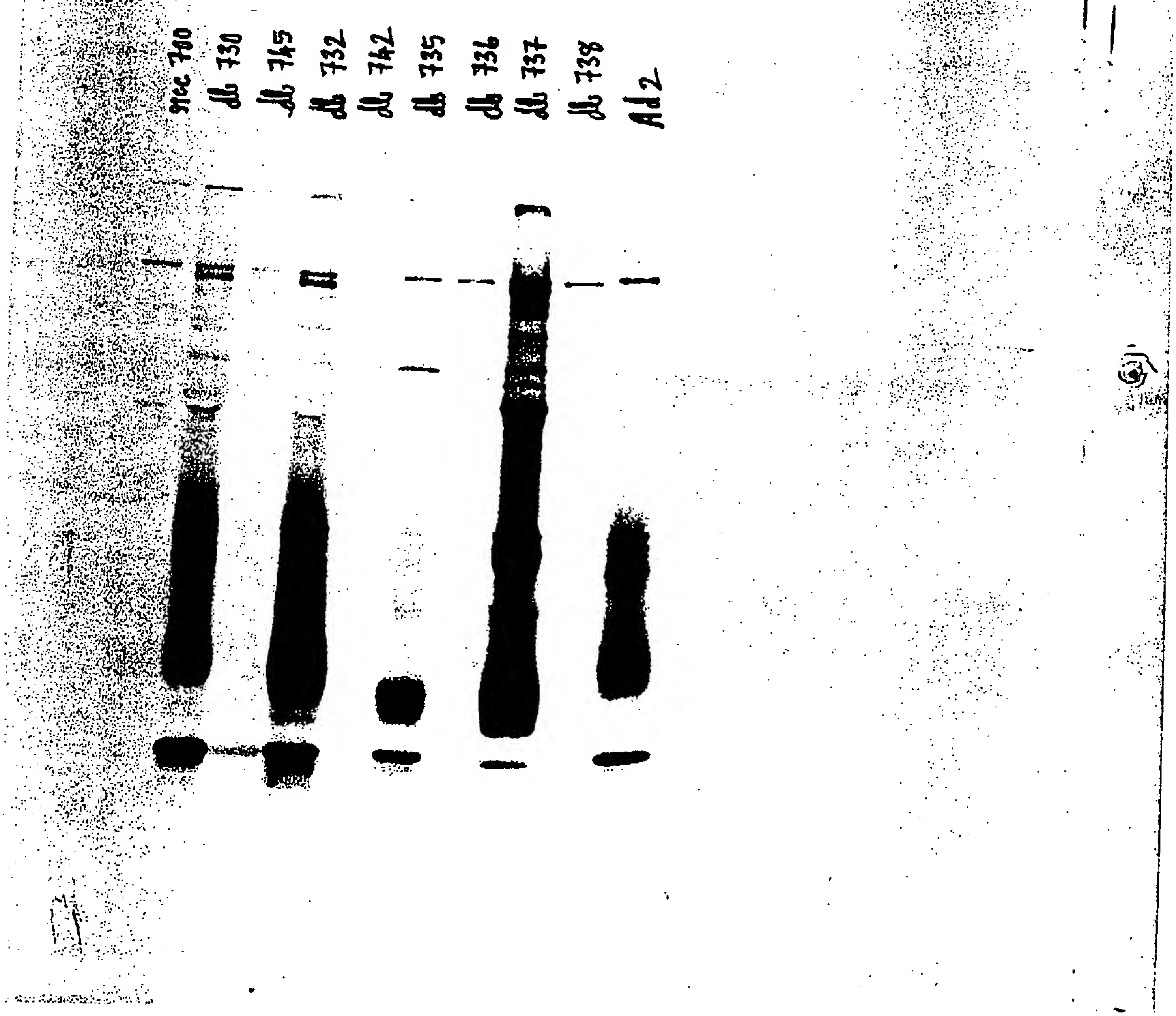


EXHIBIT
A21

Stained by A. S.

set #1		set #2		set #3	
1 mock	α11.6 (2-16) / α Fiber	13 714	α11.6 (2-16) / α Fiber	25 732	α11.6 (87-[illegible]
2 rec700		14 715		26 742	
3 dl712		15 713		27 735	
4 730		16 25mer		28 737	
5 745		17 734		29 738	
6 732		18 734/m56		30 35-mer	
7 742		19 734/m49		31 714	
8 735		20 mock	α11.6 (87-101)	32 715	
9 736		21 rec700		33 25-mer	
10 737		22 dl712		34 734	
11 738		23 730		35 734/56	
12 35-mer		24 745		36 734/49	

1st Ab's:

α11.6 (2-16) (910830 preads 2X; 1:4 dil)
#98689 bleed out

800μl [(2-16) (1:200) ← 16μl
Fiber (1:500) ← 1.6μl ← mA[illegible]

α11.6 (87-101) (910830 preads 4X; 1:4 dil)
#78686 bleed out

720μl [(87-101) (1:400) 7.2μl
712.8

2nd Ab's: [α Rabbit-FITC / α Mouse-RITC]
(all 1:50 dil's) or α Rabbit-FITC

EXHIBIT A22

slides)

920730 Descriptions of AS 11.6K Mutants (IF)

① - α 11.6K (Z-16) (1:200 dil) (1:400 α Fiber-RITC)

① L-mock - ~no backgd for Fiber; slight background for α 11.6K (esp. for mitotic spindle poles)
R-rec700 - all in late stage (by Fiber staining); ~20-30% of cells stain for 11.6K (n.e. + Golgi primarily), some "debris" or aggregated material

② L-dl712 - 100% infn; no 11.6K staining
R-dl730 - 20-30% stain for 11.6K → maybe more Golgi + debris staining; 100% infected

③ L-dl745 - ~100% infn; poss. very diffuse Golgi staining → not very bright + see no n.e. staining
R-dl732 - 70-80% stain for 11.6K → n.e., Golgi (Golgi staining may be reduced); a lot of aggregated material

④ L-dl742 - looks ~ like dl712
R-dl735 - 100% infn; very little 11.6K staining (poss a bit in Golgi, not really n.e.)

⑤ L-dl736 - 100% infn; little if any staining poss some aggreg'd material but not definitive
R-dl737 - 100% infn; bright staining; 30-40% of cells stained → n.e., debris + virus replication sites

⑥ L-dl738 - 100% inf'n; 11.6K aggregated material
R-35-mer - " " ; not so much n.e. staining + Golgi staining is very diffuse; more e.r.?

⑦ L-dl714 - ~30% of cells stained, good n.e. + Golgi but also a lot of e.r. (do not see plasma membrane)
R-dl715 - like 714 except less n.e. staining + more aggregated material

⑧ L-pm734 - like 712
R-734/msk - [illegible]

EXHIBIT
A23

Descriptions (cont'd)

(9) L- dl 713 - 50-60% stained, α n.e., Golgi + a lot of e.r. - maybe more aggregated material
R- 25-mer - less n.e. staining; seemingly more Golgi staining; seemingly less staining overall

~~(1) L- pm 734/m49 - like dl 712~~

#(11)-(20) α11.6K (87-101) (1:400 dil.)

(11) L- mock - slight Golgi-like pattern (very light)
R- rec 700 - ~100% stained, good n.e., Golgi; more e.r. + particulate than ~~sometimes~~

(12) L- dl 712 - a bit anti-nuclear (not 11.6K pattern)
R- dl 730 - ~100% of cells; very bright, maybe a bit more into all membranes (but n.e. + Golgi most)

(13) L- dl 745 - more "grainy" staining + more aggregated material, otherwise quite "normal"
R- dl 732 - almost 100% with very bright n.e. staining, also Golgi + some e.r. (brighter than rec 700)

(14) L - dl 742 - like 712
R - dl 735 - almost only Golgi (very reduced n.e.)

(15) R- dl 737 - very bright; typical pattern but more particulate + more e.r.

(16) L- dl 738 - not in membranes; some diffuse staining but mainly lots of aggregated material
R- 35-mer - bright; in all membranes

(17) L- dl 714 - very bright; in all membranes
R- dl 715 - less n.e. and more in plasma membrane (than dl 714)

(18) R- 25-mer - more Golgi, less n.e., vesicles or particles in cytoplasm

(19) L- pm 734 - fairly typical but "grainier" appearance
R- 734/m56 - sim to p 734 but not quite as bright

(20) L - 734/m49 → sim to 734, maybe more Golgi + aggregat. St.!

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Dec. 19 2002 10:43AM P5

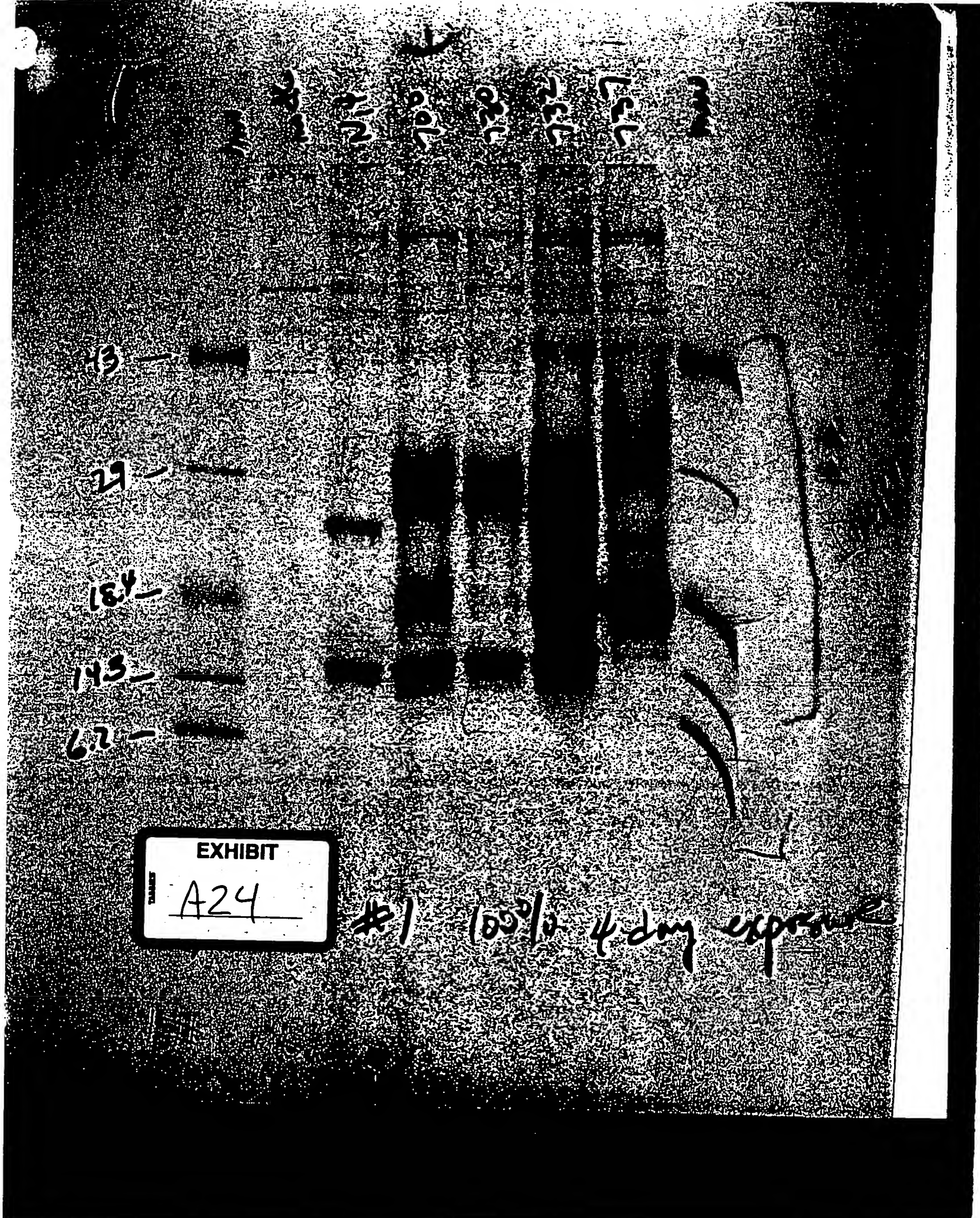
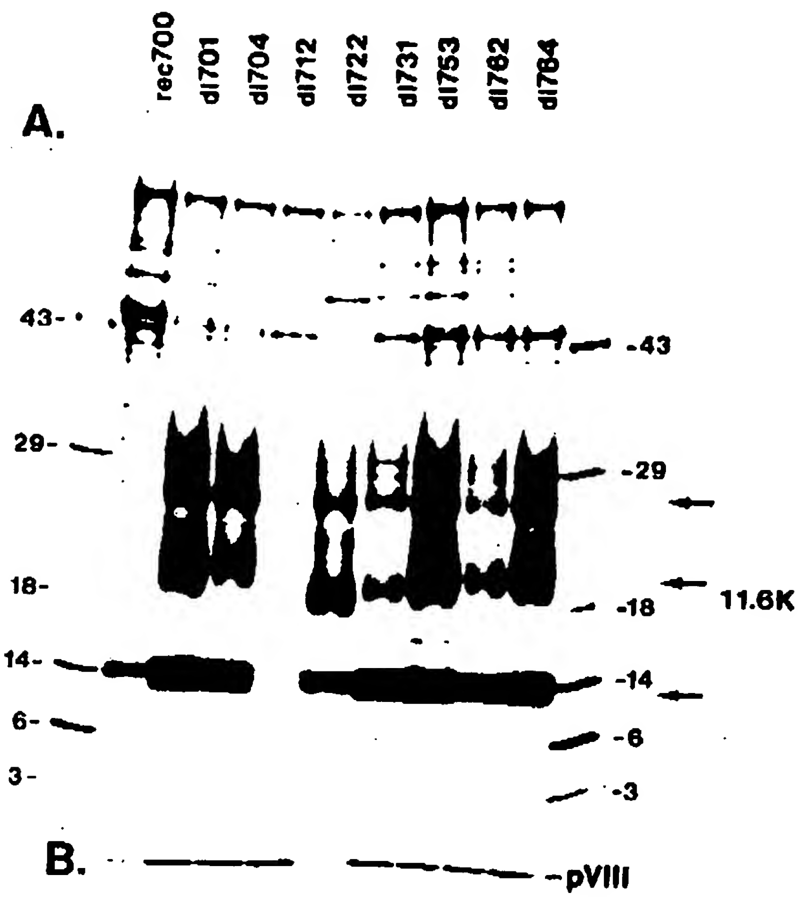


EXHIBIT
A25

IF
910827 ! prob ...?



900331 ccl #1
α 11.6K (87-101)

Have all these prints.
Fig. 1

(43)# ← (5/1) IF ... pVIII

EXHIBIT
A26

(checking for 11.6K ...)

Assays of Virus Release from A549 cells

(all .5*)

Sample #	Infection	Sample Plated cell #	pfu	Time (p.i.)	PA date	PA dilutions
1	mock	2×10^6	—	7½h	—	—
2	mock	5×10^5	—		—	—
3	rec700	2×10^6	10^5		(6/28) AT	$.5*10^0$, 10^{-1}
4		5×10^5	10^5			10^0, 10^{-1}
5		2×10^6	10^3			10^0
6	dl712	2×10^6	10^5			10^0, 10^{-1}
7		5×10^5	10^5			10^0, 10^{-1}
8		2×10^6	10^3			10^0
9		5×10^5	10^3			10^0
10	dl753	2×10^6	10^5			10^0, 10^{-1}
11		5×10^5	10^5			10^0, 10^{-1}
12		2×10^6	10^3			10^0
13	mock	2×10^6	—	24h	(6/29) AT	10^0
14	mock	5×10^5	—			10^0
15	rec700	2×10^6	10^5			10^{-1}, 10^{-3}, 10^{-5}
16		5×10^5	10^5			10^{-1}, 10^{-3}, 10^{-5}
17		2×10^6	10^3			10^0, 10^{-2}, 10^{-4}
18	dl712	2×10^6	10^5			10^{-1}, 10^{-3}, 10^{-5}
19		5×10^5	10^5			10^{-1}, 10^{-3}, 10^{-5}
20		2×10^6	10^3			10^0, 10^{-2}, 10^{-4}
21		5×10^5	10^3			10^0, 10^{-2}, 10^{-4}
22	dl753	2×10^6	10^5			10^{-1}, 10^{-3}, 10^{-5}
23		5×10^5	10^5			10^{-1}, 10^{-3}, 10^{-5}
24		2×10^6	10^3			10^0, 10^{-2}, 10^{-4}

at indicated times p.i., 1 ml of sup was removed, cells were pelleted (microfuge, 3 min) + sup removed to new Eppendorf; PA's of 0.5 ml volumes (single dishes); added back 1 ml of fresh DME (2% FCS) to each dish to replace volume removed

EXHIBIT

B

SAINT LOUIS UNIVERSITY
HEALTH SCIENCES CENTER

SCHOOL OF MEDICINE

1402 S. Grand Blvd.
St. Louis, MO 63104
314/577-8435
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Department of Molecular Microbiology & Immunology

William S. M. Wold, Ph.D.
Professor and Chairman

Dr. Rae Lyn Burke
Chiron Corporation
Department of Virology
4560 Horton Street
Emeryville, CA 94608

Dear Rae Lyn,

I have finally returned to my office and cleared off the stack of work on my desk. Thank you for providing the names of venture capitalists, and for contacting Rajen Dalal. I have decided to take the less visionary approach mentioned in your letter of June 10, 1994, and to request funding from Chiron for the gene therapy project on the adenovirus E3-11.6K protein that promotes cell death. The proposal is enclosed. I have submitted a patent application for this project; if the patent is awarded, it would provide protection in the U.S.A. but not in Europe or Asia.

Also enclosed is a budget for the proposal. I anticipate carrying out the research in my laboratory here at St. Louis University. However, other alternatives, including doing the work at Chiron, are also possible.

I have requested a consultant fee for myself and Dr. Ann Tollefson. Ann is an Associate Research Professor working in my laboratory; she is the co-discoverer of the cell death-promoting properties of 11.6K. She will co-direct the project and she will perform benchwork on the project. Note that our consultant fees are separate from the requested budget for the project.

I am also considering establishing a corporation, perhaps in August, with St. Louis University as a minor partner. If Chiron decides to fund this project, perhaps the funding can be channeled through this corporation. Therefore, the budget for the project should be considered to be tentative.

Our Biotechnology Transfer Office requires that an appropriate official from Chiron sign a confidentiality agreement. The form is enclosed.

Dr. Rae Lyn Burke

Thank you again for your interest, and for your hospitality during my visit.

With best regards,

Bill

William S. M. Wold, Ph.D.
Professor and Chairman

WSMW:jlm

enclosures

BUDGET

Personnel:	
Lynda K. Hawkins, Ph.D.	$20,700
TBA Postdoctoral Fellow	25,600
Fringes	9,955
Subtotal Personnel	56,255
Supplies:	24,000
Other:	
Maintenance contracts and repair	1,200
Telephone, postage, photocopier demurrage	400
Radiation disposal	500
Publication, illustrations	500
Subtotal other expenses	2,600
Subtotal	82,855
Indirect costs (10%)	8,286
TOTAL	$91,141
Consultant costs*	$12,000

*In addition to total project budget.

CONFIDENTIALITY AGREEMENT

THIS AGREEMENT is made by and between St.Louis University Medical Center having a principle place of business located at 1402 South Grand Blvd., St.Louis, MO 63140 and Chiron Corporation .... a .... corporation having a principle place of business located...

THE PARTIES HAVE AGREED AND DO AGREE AS FOLLOWS:

1. This AGREEMENT refers to the work of Dr............. William S. M. Wold .... relating to adenovirus 11.6K protein that functions in promoting cell death. ....

2. .... agrees to maintain the confidentiality of Proprietary Information discussed during their meetings. Proprietary Information shall not either be used or disclosed to any other person or entity without the written consent of the St.Louis University Medical Center except as may be required under a court order.

3. The confidentiality obligation shall not apply to:

   a: Information which .... can establish by reasonable proof was in its possession at the time of disclosure.

   b: Public domain information or information which after disclosure becomes part of the public domain information by publication or otherwise except by breach of this Agreement.

The term and condition herein are acceptable to both parties of this Agreement as evidenced by the signatures of their authorized representatives:

Signature______________________ Date: ____________________

Name: Aftab Alam

Title: Director, Biotechnology Transfer Research Center

Organization: St.Louis University Medical Center

Signature________________________________ Date

Name/Print________________________ Title:____________________

Organization:...............................

ADENOVIRUS E3-11.6K PROTEIN AS A CELL DEATH-PROMOTING AGENT

A. BACKGROUND

The human adenovirus type 2 (Ad2) and Ad5 E3-11.6K protein (MW of 11,600) (Fig. 1) was first identified by our laboratory, using an antipeptide antiserum to immunoprecipitate the protein from adenovirus-infected cells (Wold et al., 1984). Subsequently, we have prepared three additional antipeptide antisera, we have isolated mutants that completely delete the gene, and we have constructed a series of about 20 mutants with truncations, small in-frame deletions, and missense mutations in the gene (Fig. 2; Tollefson et al., 1992; unpublished results). Using these reagents, we have shown that 11.6K is synthesized in low amounts during early stages of infection (prior to viral DNA replication), but in large amounts at late stages of infection (Tollefson et al., 1992). We have also shown that 11.6K is an N-linked O-linked integral membrane protein that initially localizes to the endoplasmic reticulum (ER) and Golgi apparatus, but ultimately localizes to the nuclear membrane (Scaria et al., 1992).

We have recently discovered that the 11.6K protein functions to promote cell death (A.E. Tollefson, A. Scaria, and W.S.M. Wold, manuscript in preparation). A representative experiment is shown in Fig. 3. As measured by release of lactate dehydrogenase (LDH), cells infected with wild-type adenovirus (*rec*700) begin to die at 2-3 days postinfection (p.i.), and are mostly dead by 5-7 days p.i. In contrast, cells infected with a mutant (*dl*712) that deletes only the 11.6K gene stay completely alive until 5 days postinfection, and they do not begin to die until 6 days p.i. Similar results have been obtained using, as indicators of cell death, the release of adenovirus from infected cells, trypan blue exclusion, the MTT assay (which measures mitochondrial activity), DNA degradation (agarose gels, DAPI staining, Apo-Tag), and light and electron microscopy. We believe that the function of 11.6K in adenovirus biology is to lyse

cells, thereby allowing adenovirus to be released from the infected cell.

Using our collection of virus mutants in the 11.6K gene, we are currently performing a structure-function analysis of 11.6K. Some of the mutants are shown in Fig. 2. The phenotypes of the mutants are summarized at the right side of Fig. 2. SDS-PAGE analysis indicated that all the mutant proteins are stable with the possible exception of *pm*734.1 which only synthesizes residues 49-101 of the 11.6K protein (data not shown). These mutants have allowed us to map the protein domains required to target 11.6K to the nuclear membrane, and for 11.6K to promote cell death. Briefly, residues 41-59, the only hydrophobic domain in the protein, serves as the signal to insert 11.6K into membranes and to anchor 11.6K within membranes, i.e., this domain is a signal-anchor sequence. Residues 63-78, which includes the basic-proline domain (residues 63-74), a domain rich in basic amino acids and proline, is required to target 11.6K *specifically* to the nuclear membrane; mutant proteins that lack these sequences localize to *all membranes*, not specifically to the nuclear membrane. Regarding cell death, residues 1-40 and 71-101 can be deleted without abrogating the ability of 11.6K to promote cell death. Thus, the "death domain" appears to consist of residues ca. 46-60, with help from residues 61-74. However, we emphasize that although we have shown that these sequences are necessary to promote cell death, we have not shown that they are sufficient.

We do not understand the mechanism of action of 11.6K in targeting to the nuclear membrane and in promoting cell death. We believe that the nuclear membrane is the site of action of 11.6K because certain mutant proteins that do not exit from the ER or Golgi are defective in promoting cell death. Future studies in the laboratory will focus on the mechanism of action of 11.6K. This mechanism is not only of fundamental interest, but it may also

elucidate the cellular mechanisms that control cell death; this latter information may allow for novel gene therapy approaches for killing or protecting cells.

B. PROPOSAL

Since the 11.6K protein can promote the death of adenovirus-infected cells, it has the potential use as a therapeutic agent to kill cells, e.g. malignant cells, in humans. This proposed research addresses two issues that must be resolved in order for 11.6K to be used as a therapeutic agent.

First, although we know that 11.6K promotes the death of *adenovirus-infected cells,* we do not know whether 11.6K can *function autonomously* to kill cells.

Second, since 11.6K functions inside the cell, apparently at the nuclear membrane, a means must be developed to deliver the protein to the cells of interest.

C. EXPERIMENTAL PROCEDURES

C.1. Can the 11.6K protein function autonomously to kill cells?

In order to answer this question, we have attempted to isolate human A549 cells stably transfected with the 11.6K gene. Several neo^R-resistant cell lines have been obtained. These cells express only low levels of 11.6K, and the 11.6K protein is localized in the Golgi but not the nuclear membrane. Since we were not able to obtain cells that express high levels of 11.6K, or cells wherein 11.6K is localized to the nuclear membrane, these experiments suggest, but do not prove, that 11.6K can function autonomously to kill cells. We propose two approaches to address this question in more detail.

C.1.a. *Tet*-inducible vector.

We have obtained the *tet*-inducible vector system from Hermann Bujard

(Gossen and Bujard, 1992). In the *tet* system, the tetracycline repressor (tetR) was fused to the transactivation domain of VP16. In the absence of tetracycline, the fusion protein will bind to *tet* operators and will transactivate a promoter consisting of *tet* operators plus a TATA box. In the presence of tetracycline, the tetR-VP16 fusion protein does not bind to *tet* operators, and the promoter is silent. The major advantages of this system are that, upon removal of tetracycline, the gene is induced by up to five orders of magnitude. Also, induction is rapid, being >20% in 4 h and 100% in 12 h. Finally, the low amount (0.1 μg/ml) of tetracycline required to keep the gene silent is unlikely to affect other properties of the cell. We will clone 11.6K into the *tet* vector, develop cell lines, and examine whether 11.6K is induced by removal of tetracycline, and whether 11.6K promotes cell death following induction. Cell death will be measured by release of LDH, trypan blue exclusion, the MTT assay, and microscopy.

If 11.6K alone does promote cell death, then we will proceed with the experiments described in Section D below. If 11.6K alone does not promote cell death, then we will proceed with the experiments in Section C.3.

C.1.b. Adenovirus vector.

There are two general classes of adenovirus vectors, nondefective and defective for replication in cultured human cells. The nondefective vectors generally have the E3 transcription unit (Fig. 4) deleted and replaced with the transgene. The E3 genes are not required for virus replication in cultured cells or in the lungs of hamsters or cotton rats, so these vectors are able to replicate. However, the E3 genes function to block the immune and inflammatory response to virus infection, so the E3-deleted vectors are more pathogenic than wild-type adenovirus.

Defective vectors generally have the E1A and E1B regions deleted and replaced with the transgene, and they have the E3 region deleted in order to increase the amount of foreign DNA that can be inserted into the viral genome (only 105% of the genome can be packaged). The E1A and E1B genes (E1B-55K) are essential for virus replication. E1A proteins are required to efficiently induce transcription of the other adenovirus transcription units. The E1B-55K protein is required for efficient transport of viral "late" mRNAs from the nucleus to the cytoplasm. Since the defective vectors lack E1A and E1B, they cannot replicate in ordinary cell lines or in animal models. The vectors can, however, replicate well in 293 cells, a human cell line that provides the E1A- and E1B-encoded proteins *in trans*.

Although E1A is required for *efficient* induction of transcription of the other adenovirus transcription units, transcription of these genes, including late genes, can occur at low levels in the absence of E1A. Indeed, recent animal model studies and Phase I human trials have indicated that defective adenovirus vectors do elicit an inflammatory response, presumably due to low level expression of adenovirus proteins.

Since we eventually hope to design an adenovirus vector to promote cell death, it will be important to limit the infection *in vivo* to the target tissue, and to minimize infection of healthy tissue. Therefore, the vector should probably be defective. The expression of the 11.6K protein could be limited to the tumor by direct injection into the tumor, or by use of a tumor-specific promoter to drive expression of the 11.6K gene. It is unclear whether the E3 genes should be included to minimize the normal inflammatory response (probably not), or excluded in order to maximize the response; this presumably would depend on the amount of infection of healthy tissue.

The design of an optimal vector is a topic of future experiments. At the present, we will use a simple "first generation" vector to address whether 11.6K expressed essentially alone can kill cells. This experiment differs from that described in Section C.1.a. because the vector will express other adenovirus proteins at low levels.

Our vector will have an Ad5 backbone, and it will be deleted in the E1A, E1B, and E3 regions. The 11.6K gene will be inserted into an expression cassette wherein transcription will be driven by the cytomegalovirus immediate early promoter, and the pre-mRNA will be processed using SV40 polyadenylation and splicing signals. The expression cassette will be inserted into the E1A/E1B region (Fig. 4), and plaques will be picked on 293 cells. Plaques of vector expressing 11.6K should be larger (more cell lysis and virus spread) than plaques from vector lacking 11.6K. Plaques will be expanded into virus stocks, and high-level expression of the 11.6K protein will be confirmed. Cultured human and mouse cells will be infected with the vector, and cell death will be monitored as described in Section C.1.a.

If 11.6K expressed from the adenovirus vector kills cells, then we will proceed with the experiments in Section D. If cells are not killed, then we will perform the experiments in Section C.3.

C.2. Mapping the minimal domain of 11.6K that is sufficient to promote cell death.

Our studies with 11.6K mutants have indicated that a protein consisting only of residues 41-101 (i.e. lacking residues 1-40) localizes quite efficiently to the nuclear membrane and is about 50% as effective as the wild-type 11.6K protein in promoting cell death. A protein lacking residues 79 to 101 is completely wild-type for localization to the nuclear membrane and promotion of cell death. These results suggest that a version of the 11.6K protein consisting

only of residues 41-78 might be sufficient to promote cell death. It is important to determine whether this is so, because such a peptide, synthesized *in vitro*, might by itself be sufficient to promote cell death (e.g. by injecting the peptide into tumors). Also, such a peptide might be less immunogenic than the entire 11.6K protein. We propose to construct an adenovirus mutant that expresses residues 41-78 and determine whether this protein can promote cell death. This mutated gene will be inserted into the *rec*700 background, as we have done for all our other 11.6K mutants, and it will be expressed from the *tet*-inducible vector.

C.3. <u>What is required in adenovirus-infected cells in order for 11.6K to promote cell death</u>?

If the experiments described in Section C.1. indicate that 11.6K alone cannot promote cell death, then some other adenovirus gene product or some unknown aspect of the adenovirus productive infection must be required in order for 11.6K to function. The following experiments will be performed to address these issues.

First, we will determine whether 11.6K overexpressed during early stages of infection can promote cell death. The 11.6K gene will be built into our mutant, *dl*7001, which lacks the entire E3 region but expresses all other adenovirus genes. We are using this mutant as a vector to express the E3 proteins individually (Ranheim et al., 1993). Cells will be infected with the *dl*7001-11.6K vector, maintained in cytosine arabinoside in order to inhibit viral DNA synthesis and keep the cells in the early stage of infection, and cell death will be monitored.

Second, we will attempt to map the adenovirus early protein(s) that must collaborate with 11.6K in order for 11.6K to promote cell death. We will infect cells with mutants that lack the E4 region, the E1B region, and the E1A region. We already know that

this putative "collaborating" protein is not encoded by the E3 region. These experiments are similar to those we performed to map genes that function to sensitize cells to tumor necrosis factor (Duerksen-Hughes et al., 1989) and to down-regulate the epidermal growth factor receptor (Carlin et al., 1989). If we determine that an adenovirus late gene encodes the "collaborating" protein then we will attempt to map the gene using available temperature-sensitive mutants.

D. POTENTIAL FUTURE EXPERIMENTS

The following is a brief description of possible future experiments. Which experiments will be attempted will depend on the results of the experiments in Section C. Most or all of these experiments will probably require additional funding.

D.1. Design of an optimal vector to deliver the 11.6K protein to cells.

We will attempt to optimize expression of 11.6K in our adenovirus vector, and we will construct vectors with tissue-specific promoters. We will explore whether the E3 region should be included in the vector, and whether a nondefective vector might be useful.

We will also attempt to express 11.6K from a retrovirus vector. Since retrovirus vectors only infect dividing cells, such a vector that expresses 11.6K might be particularly useful to treat brain tumors.

D.2. Delivery of the 11.6K protein by direct injection.

Since 11.6K has an internal signal-anchor sequence and it has only a short sequence that is C-terminal to the signal-anchor domain, 11.6K may be able to insert into membranes in a posttranslational manner. If so, then liposomes could be an ideal mechanism to deliver the protein to the interior of cells. The 11.6K protein might integrate into the

membrane of the liposomes, become fused to membrane vesicles within cells, and then be targeted to the nuclear membrane via its specific nuclear membrane localization signal. We will attempt this experiment using 11.6K purified from adenovirus-infected cells, where 11.6K is made in large amounts. Alternatively, 11.6K could be obtained from bacterial or baculovirus vectors.

If the experiment described in Section C.2. results in the identification of a minimal domain for 11.6K to function, then the minimal peptide will be synthesized *in vitro*. The peptide will either be incorporated into liposomes or added directly to cells, and cell death will be monitored.

D.3. Coupling of the 11.6K protein to a ligand.

In one of our adenovirus mutants (*dl*718), the stop codon for 11.6K is deleted; this results in the synthesis of a fusion protein consisting of residues 1-87 in the 11.6K protein fused to residues 14-91 of the E3-10.4K protein. This fusion protein functions nearly as well as wild-type 11.6K in promoting cell death. This suggests that 11.6K could be fused at its C-terminus to some ligand, e.g. EGF, and still remain functional. If so, then the ligand could be used to target 11.6K to specific cell types that express the receptor for the ligand. Thus, the experiment would be to express the 11.6K-ligand fusion protein in bacteria or insect cells, purify the protein, add the protein to the cells, and determine whether the protein is efficiently internalized into cells that express the ligand receptor, and whether the protein promotes cell death.

D.4. Animal models.

We have found that 11.6K does not localize to the nuclear membrane in

adenovirus-infected mouse or rat cells. This may imply that 11.6K must interact with a human protein in order to promote cell death. Lack of 11.6K function in mouse or rat cells is in one respect unfortunate because an animal model will be necessary to determine whether 11.6K can, for example, cause regression of tumors *in vivo*. We will infect cells of different species, e.g. cotton rat, hamster, and monkey, and determine whether 11.6K localizes to the nuclear membrane and promotes cell death. It may also be possible to examine human tumors growing in immunoincompetent mice. When we find a species where 11.6K is able to promote cell death, then experiments will be done in this animal model.

REFERENCES

Gossen, M., and Bujard, H. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* 89, 5547-5551.

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Scaria, A., Tollefson, A.E., Saha, S.K., and Wold, W.S.M. 1992. The E3-11.6K protein of adenovirus is an Asn-glycosylated integral membrane protein that localizes to the nuclear membrane. *Virology* 191, 743-753.

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that encodes an 11,600-molecular-weight protein in the E3 transcription unit of adenovirus 2. *J. Virol.* 52, 307-313.

FIGURE LEGENDS

Fig. 1. Sequence of the Ad2 E3-11.6K protein. The protein becomes integrated into membranes via its internal signal-anchor domain at residues ca. 41-59. The protein is oriented in the membrane with the NH_2-terminus in the lumen and its COOH-terminus in the cytoplasm or nucleoplasm (the protein ultimately localizes to the nuclear membrane, but we do not know whether it is the inner or outer nuclear membrane). The site of N-glycosylation is indicated (NTT), as are the major sites of O-glycosylation. A domain rich in basic amino acids and prolines is indicated.

Fig. 2. Schematic representation of the Ad2 11.6K protein, as well as a subset of the mutants constructed in the 11.6K protein. For the wild-type protein (top figure), the major and minor O-glycosylation sites are shown, the N-glycosylation site is shown, the methionines at positions 1, 41, 49, and 56 are shown, the signal-anchor domain is shown, and the basic-proline domain is shown. Also shown is the orientation of the protein in the membrane, with the NH_2-terminal region in the lumen of membranes, and the COOH-terminal region extending into the cytoplasm and/or the nucleoplasm. The deletions shown for the various mutants are indicated by the schematic. The two right columns in the figure indicate the approximate phenotype of the mutants with respect to their ability to promote cell death, as a percentage of the wild-type protein, and their ability to localize to membranes. Membranes shown in italics are the major sites of localization. NM refers to nuclear membrane, G refers to Golgi. The protein depicted at the bottom of the figure corresponds to the putative proteolytic processing product that arises following initial synthesis of the 11.6K protein.

Fig. 3. A cell viability assay for cells infected with *rec*700 (wild-type) or two mutants,

*pm*712 and *pm*734.1, which lack a functional 11.6K gene. A549 cells were infected with the viruses, then at different days postinfection cell lysis was measured based upon the release of lactate dehydrogenase (LDH) into the culture medium. The experiment shows that cells infected with *rec*700 die (i.e. release LDH) much more rapidly than do cells infected with the two 11.6K mutants.

Fig. 4. Schematic of the Ad2 genome. The genome is a linear duplex DNA of 36,000 base pairs. *r* and *l* refer to rightward and leftward transcription, respectively. The split arrows indicate the spliced structures of the mRNAs (exons are shown). 289R, 19K, IIIa, etc. refer to proteins. E1A, E1B, E2 (A and B), E3, E4, and L1 (early) are "early" transcription units that are expressed prior to viral DNA replication. L1-L5 are families of "late" mRNAs expressed in the major late transcription unit. The 11.6K protein is translated from one of the E3 mRNAs at early stages of infection. At late stages of infection, 11.6K is translated from an mRNA that contains the major late tripartite leader (Tollefson et al., 1992).

EXHIBIT

C1

Kostya, January 30
Project: Develop replicative vectors for cancer gene therapy.

Progress achived:

1) 3 rounds of transfection were performed with p626 (pBHG11 with E4 promoter substituted for for CMV promoter). No plaques were obtained with this construct or pBHG11 as a control. I have prepared new stock of pAV56 (left-arm plasmid) and repeated the experiment 3 times more. Now waiting for results.

2) The plasmid containing E4 promoter- SPB promoter substitution was constructed (p82). It contains DNA of dl309 from BamHI to NdeI site (E3 region from dl309) and other part to the end of genome from pBHG11 with substitution of E4 promoter. 1 cotransfection experiment was done recently with 1101/1107 cut with EcoRI and pCMV/TTF. Get none TTF [illegible]

3) Cotransfection of 1101/1107 cut with EcoRI and p54 (Lynda's plasmid pdlLKH Bam-end with insertion of Ad5 ADP gene) was done. Up to now (26 days) there are no plaques with either 1101/1107/ EcoRI or with 1101/1107/EcoRI+ Lynda's plasmid (parental plasmid without insert of ADP). With p54 there are 7 plaques. The plaques have been picked up and initial stocks of viruses prepared. Now the work is in progress to infect monolayers and characterize the genomes of this viruses. appear in 2 weeks.

4) I failed 2 times to construct the large plasmid with mutated gp19K on dl309 background. Now repeating the cloning.

[illegible] ADP inserted in XbaI site. Not start in TP.

Proposed work:

1) To join Carol's and Mohan's work on 1101/1107 and pm975. I presume it would be interesting to do the same work on primary lung epitelial cells (GTI). SAEC - normal human lung small airway epithelial cells. GTI get cells from GTI.

2) Start transfection experiments on Mohan's cell line (293 transformed with pCMV/TTF) to obtain the virus with E4- SPB promoter substitution. Characterize Mohan's 293-TTF cells on their ability to transactivate SP-B promoter (transfection and IP with anti-E4 ORF6 antibodies).

3) Start the work to obtain the plasmid with E1 on 1101/1107 background? Make the point mutation as in pm975? Try to substitute E1 promoter for SPB? Enlarge the CR2 deletion?

GTI - infect cell lines.

Jan 31, 97

1

Outline of Mohan's project:

1. C.F. project:

a) plasmid pΔE1spl A / 3.7 SPC Rep78 has ~2900 bp deletion. (Gautum's plasmid).

b) Construction of pΔE1 spl A / 3.7 SPC Rep78 is in progress: Transfering the 3.7 SPC/Rep78 fragment to pΔE1 spl A plasmid. Next week SPB/Rep78.

c) Preparation of βgal assay. high titer BHG E3 ITR LacZ virus. 1.4×10^9 pfu/ml. Done with 40 dishes.

Looks like virus does not grow well.
Try again in KB cell/E1 growing in spinners.

KB/E1 - looks like may grow in spinner.

EXHIBIT
C2

2. Cancer project:

a) Experiment 1:

a-1): Growing HEL 299 cells infected with dl 1101/1107 Showed CPE ~ 12 days.
dl 309 showed CPE on 3rd day.

a-2): Growth arrested HEL 299 Cells infected with dl 1101/1107 did not show CPE, till 17th day. dl 309 showed CPE on 8th day

a-3): IF showed strong presence of fiber and DBP in ~ 50% of the growing cells (infected with dl 1101/1107) on 14th and 17th day.

b) <u>Experiment 2:</u>

b-1): pm 975 infected growing HEL 299 cells showed CPE on 3rd day; dl 309 also showed CPE on 3rd day.

: pm 975 and dl 309 killed all the cells ~ 6th day.

, dl 1101/1107 Showed CPE begining ~ 12-14th day:

b-2): Till 10th → 17 days — still still no CPE day no visible CPE was seen cells frozen. Next week plaque assay. in pm 975 and dl1101/1107 infected growth arrested cells.

dl 309 showed CPE on 8th day.

b-3): Growth curve = Growing and growth arrested viral infected cells are being frozen for plaque assay.

EXHIBIT

63

c) Experiment 3:

c-1) HEL 299 Growing cells are infected with pM975; dl 1101/1107: dl 309 and mock, at MoI of 100 pfu and 500 pfu/cell.

3rd of infection, at 100 pfu pM975 showed Moderate CPE and ~90% CPE in 500 pfu infectio

No CPE CPE is seen in dl 1101/1107 infected cells at 100; and 500 pfu/cell. (7th day)

dl 309 showed CPE on 3rd day (100 pfu) and 90% CPE in 500 pfu infection.

Growth arrested cells will be infected today.

Plaque assay next week.

EXHIBIT
C4

Gene Therapy - Group Meeting 970241

Took 1 h 20 min

Karoly 2/07/97

Did not make ~~Nrippo~~ 14.7 in RID, E1A

E3 viruses

- I have made CsCl stock of βGluc (D-) 14.7 and NPGK pp19K ap
- titering is under process (N^nrippo PGK pp19k ap ≈ 10^9)
- going to make Ad lac Pol RID and Ad lac Pol 14.7

RID in E3 region. No band in CsCl.

Bai - CMV-14.7 CMV-RID. Have clone. ~~Make virus.~~

FasL project

~~FasL~~ co-transfected 293 10.4/11.5 plasmid pCI (Bai made) cells died.

- I have cloned mouse FasL in pCI, then the CMV-mFL-PolyA casette in a left end plasmid containing β-Gal and VARNA I-II. cells die very fast
- Transfected this construct in 293 → the cells died
- I could not show mFL in a transient transfection assay (293 cells, Westernblot with anti human FasL) but the protein could be detected in an in vitro transcription - translation reaction using the same plasmid and antibody
- Transiently transfected MCF-7 and MCF7 Fas cells die at the same rate. No cyclo treatment is needed
- I am going to try MCF-7 CrmA first in transient transfections, then make a cell line. This cell line can be used as an „activated CTL" in Fas experiments
- I am making ~~a~~ CrmA-293 line

Cancer therapy project

- It seems that the WI38 I use is too high passage. Only a proportion of the cells divide, the majority is senescent. I have ordered a new batch from ATCC.

EXHIBIT C5

February 14, 1997 Kostya

1) Up to now I have no plaques after transfections with p626 (E4 → CMV promoter substitution). And I have no plaques with pBHG11 as a control.

2) I am preparing now DNA from cells infected with 10 plaques resulting from cotransfection of 1101/1107 DNA cut EcoRI with p54 (Lynda's deletion of E3, insert of ADP gene).

3) I have plaques after cotransfection of 1101/1107 DNA cut EcoRI with p82 (E4 → SP-B) promoter substitution. Control (1101/1107 cut with EcoRI) to experiment ratio is 3:10 now ~~but~~ in dishes with p82 plaques are still appearing (3 weeks).

4) I am working on Mohan's cells (293 CMV/TTF) have done one cotransfection of 1101/1107 / EcoRI p82 on these cells.

5) Cloning experiments to obtain large plasmid suitable for cotransfection with gp19K mutated are in progress.

EXHIBIT

C6

Feb 14, 97

Outline of Mohan's Project:-

- Rdh analysis of Rep78 antisense
- ~~Hirt - photograph of passage~~ (1x4 x 10^9) 80 dishes titered on 293 cells.

1 C.F. project:

a) Screening colonies ~~for~~ 3.7SPL/Rep78 insert in pΔE1spIA.
36 Colonies are under screening: Some colonies have the insert: Screening more colonies & Characterization

b) SPB promoter = PCR; strategy to clone in front of Rep78.

Mohan - KB/E1A spinner - doing ok. Still in tube (60 ml). Not in spinner yet. 293 suspension culture. Grow slow. Forms clumps at 5x10^5 cells/ml.

2 Cancer project:

Experiment #3:

Growing HEL 299 cells: 14th day (2/14/97)

a) pm 975 (100 & 500 pfu) Killed all the cells.
b) dl 1101/1107 (100 pfu) v CPE starts; in 500 pfu - shows CPE. (better than 100 pfu)
c) dl 309 (100 & 500 pfu) killed all the cells.

Growth arrested HEL 299 Cells: 7th day

a) No CPE in dl 1101/1107 (100 & 500 pfu);
b) No CPE in pM 975 (100 pfu);
CPE shows in 500 pfu. Same as 309 at 500 PFU.
c) No CPE in dl 309 (100 pfu)
CPE shows in 500 pfu.

Growth Curve:-
Dishes are processed; Going to do plaque assay

EXHIBIT
C7

Karoly 2/14/97

E3 viruses

- Titer of N PGK pp19kexp is 1.37×10^9
- β Gluc (D-) 14.7 is less than 10^7 (?) (Will repeat titer expt.
- We are trying to make virus with the plasmids made by Bai
- We are assaying for expression of 14.7 and RID in a Western

FasL project

- I tried the infection-transfection experiment twice. I failed in both attempts because the virus killed the cells before I could transfect. I used 50 pfu/cell (293), 20 µg/ml AraC. The cells were rounded up after 18 h (1st exp.) and 7 h (2nd exp.) 5 PFU. MCF7 cells. FasL supernatant. 293/CrmA cell line.

Cancer therapy project

I have got WI38 from ATCC, I am growing them up. Got cell proliferation kit - stain for BrdR.

EXHIBIT

C8

02-21-97

Kostya

1) I have made and analyzed by restriction and PCR ~~[illegible]~~ Hirt DNA preps from 5 of 10 plaques that I had after p54 (Linda's plasmid + ADP) cotransfection with 1101/1107/RI. 2 plaques were wt with addition of something that I suggest is defective version of recombinant virus. 3 other plaques contain E3 from p54.

) I'm picking up plaques resulting from transfection of p82 (E4 → SP-B promoter substitution).

Have 10 plaques - half small, half very small. One is with. The transfection was complicated. co-transfected Ade-cut [illegible] also transfected TFF1.

Expt is being repeated - expt is 3 weeks old; Had same plaques.

3) Failed to construct large form of gp19K * plasmid due to contamination of competent cells with something AmpR. Now prepared new batch of competent cells, about to repeat cloning.

Ad/ADP recombinant on "Lynda's plasmid, a E3, +[illegible], deletion extending like p3H011.
Has 4 plaques, 2 are recombinant (Hirt, PCR), 2 seem to be mix 1 wt and recombinant with a deletion. These plaques give strange appearance, as if they are secreting a factor that kills surrounding cells - [illegible]

Lynda's plasmid - not even sure that E4 is present.

Kostya - will plaque-purify the "mutant" and "mixed" plaques.

The two mutant plaques are very "slow" ∴ may not be good killer virus.

EXHIBIT C9

Gene Therapy Group Meeting 990221

pCI — CMV-intron-gene-SV40 polyA

Karoly 2/21/9

E3 viruses

- Western with the pCI RID (CMV-RID) and CMV-14.7 plasmids (in pAE1sp1A) was negative — I am doing an IP

These are the constructs that Bai made.

FasL project

- The shedded mFasL is not toxic
- I am trying to make virus with mFasL in left-end plasmid and dl7001

Killing by FasL is very fast.

It is known that the shedded human FasL is toxic, the mouse FasL is not.

All MCF-7 cell lines die when transfected c̄ vector/FasL except the one c̄ CrmA.

They die in one day.

~30-50% of the cells die.

Will do expt c̄ MCF-7/14.7K cell line.

Terry - any MCF-7 cell line expressing RIDα or RIDβ?

Could try to repeat this expt in A549, even if transient transfection is poor.

Karl - making 293 cells expressing CrmA.

MCF-7/CrmA/FasL - have clones.

- transfect c̄ RID + LDNGFR, purify, then transfect c̄ FasL.

EXHIBIT

C10

Mohan's project.

Feb 21, 97

1. CF. Project:

a) Final plasmid pΔE1spIA/3.7 SPL Rep78 has been Constructed; plasmid preparation is under progress.

2. Cancer project:

Experiment #3:

Growing HEL 299 cells 21st day

a) dl1101/1107 shows better CPE in 500 pfu than 100 pfu.

b) pm975 & dl 309 killed the cells.

Growth arrested: 14th day

a) NO CPE in pm975 (100 pfu).
At 500 pfu at 14th day cells are killed.
BPE starts at 3rd day in localized areas. Cells dead at 12 days.

b) NO CPE in dl 1101/1107 (100 & 500 pfu)

Growth Curve:-

pm 975 - infected G & GA. HEL 299 cells:
Growth Curve is done; waiting for plaques.
Will need to do growth curve for dl1101 - will do next week.

Northern blot will be done for Rep78 antisense cell line
Trying to clone SPB/Rep78 into p.

12-28-91 Kostya

Need to do Hirt on some of those plaques.

) I have analyzed by PCR 8 of 10 plaques which I had after 1101/1107 cut with EcoRI + p82 (E4 → SP-B promoter substitution, E3 from dl 309). They all contain wt E4 prom. And I have no up to now plaques with p626 (E4 → CMV promoter substitution E3 from pBHG11). Determine the sequence of Knippan's plasmid.

) I am going to put different variants of E3 that I have in ~~other~~ small plasmids (dlE3 X60 + ADP, dl 309 with gp 19K mutated, wt?) into Linda's plasmid with which I have obtained a recombinant virus.

) I would propose to start the work toward E1 promoter deletion and substitution with SP-B or any other tissue-specific or tightly regulated promoters. As alternative we can try to substitute E2A promoter.

) Growing up ADP mutants Vectors.

BLAST lipoxygenase -3 diff species.

EXHIBIT C12

Mohan, Feb 28, 97,

1 C.F. Project:

a) Preparation of pXEIsplA/SPC Rep78 is progressing. Some problem in CsCl banding. Preparing another prep. Saw 4 bands - good band.

b) Construction of SPB-Rep78 plasmid.
: partial digestion of p3.7SPC/SV40 with Hind III -

EXHIBIT
C13

2. Cancer Project:

~~Experiment~~ # 3:
Growing HEL 299 cells 28th day

a) dl 1101/1107 shows CPE in 500 pfu and 100 pfu

1101/1107 good CPE at 500 pfu

Growth arrested cells:- 21st day.

a) pm 975 at 100 pfu shows ~ CPE?
b) No CPE in dl 1101/1107 (100 & 500 pfu)

Growth Curve:- pm 975 100 pfu. On 293 cells.

Growing: Total cell + supernatant
3rd day = 7.1×10^7 pfu/ml
5th = 1.3×10^7
6th = 1.1×10^7
7th = 7×10^6

Growth arrested:
Need to check 1st & 3rd day.
10th day = 1.2×10^5 pfu/ml
14th = 3.2×10^5
19th = 1.8×10^5

1101/1107 assay has been started. Need to stain next week.

KB/E1A and KB/neo
Growing. G418 resistant. Best proved than E1A cells.
293 cells - Froze from Marcobot.

Karoly 2/28/9

E3 viruses

3 transfection going. No plaques yet 1, 10, 18 day pi BHG11 ō BHG10 TAY ō

HG10-plaques on 16, 18 day

- IP with Bai's constructs: I could IP 14.5 (and co-IP 10.4?) from an in vitro translated sample. After one day exposure the transfected samples did not show up. The 14.7 plasmids were negati even in the in vitro translated samples. Sequencing is under process to 14.7K

- I made CsCl stocks of Nripen's two plasmid β-Gal-PGK14.7ap and β Gal RID ap. I am going to try to make virus with them.

Fas L project

Have MCF7-Fas ... clones.

- I have transfected ~~yesterday~~ RID and mFasL ~~this morning~~ (both in pCI vector) in 293 cells. Result is due this evening ~~tomorrow~~ morning

- I have infected 293 cells with the CPE stock from the dl7001 infection - LE mFL transfection experiment.

Cancer therapy project

- I use the new WI38 cells for a new round of infection with 1101/1107

I need to call Bruce Trapnell. What is real status of money.

EXHIBIT C14

3/7/97 Kostya

1) Cloning of ITR PCR product to p82 (E4-SPB promoter plasmid) is in progress.

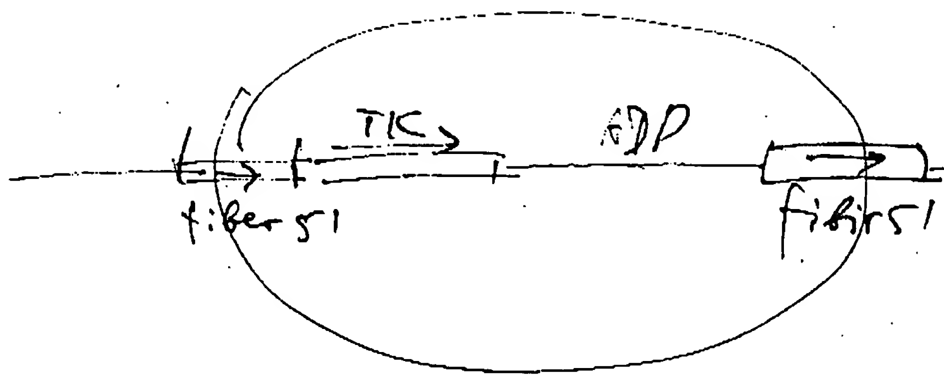
2) I have a problem with TK, cannot PCR it from Linda Morrison's plasmid. If it is possible, I would like to have a plasmid with HSV-1 TK gene from GTI.

3) Plaque assay of 544 (virus with Lynda's E3 deletion + ADP insert). on 293 cells.

309, 1101/1107 (wt Control) Lynda's deletion 12.5K, ATG mutate L4 polyA retain

544 - develops plaques a bit slower than wt, plaques appeared 1-2 days later than in wt, day 16 - plaques are ~ 8-10 mm in diameter.

7001 - plaques are tiny (1-2 mm) at day 16.



03-07-97
Kostya

1) i) Results of cotransfection 1101/1107 / EcoRI + p54 (dl E3 + ADP) → 4 more plaques were analyzed. 3 of them contain recombinant genomes.
ii) Subcloning of dl 309 E3 with gp19K mutated and dl E3 X6a + ADP into Linda's plasmid is in progress. When the plasmid with 19K * is made I'm going to insert HSV-1 TK into Pac I site created in gp19K gene.

2) i) No plaques with p626 (pBHG11 E4 promoter → CMV promoter). No plaques with pBHG11 as a control.
ii) 4 more plaques resulting 1101/1107 / EcoRI + p82 (E4 → SP-B promoter) were analyzed. All contain wt genomes.
iii) In 2 repetitions of cotransfection 1101/1107 with p82 (E4 → SP-B promoter) no plaques neither in control no in experiment. 2nd experiment was done on Mohan's 293/CMV-TTF cells.
Now repeating the cotransfection.
iv) Sequencing of Nippon's plasmid p21 (E4 promoter dl) is in progress to ~~prove~~ that right Ad ITR is intact in the plasmid.

3) Growing initial stocks of the recombinant virus with dl E3 + ADP.

EXHIBIT
C16

Kathy 3/7/97

E3 viruses
- I have transfected β-Gal PGK 14.7 ep and β-Gal PGK RIDα (with BHG11) I am waiting for plaques.
Still no plaques c̄ Bai's constructs. Now 3 weeks old.

FasL project
- The pCI RID construct did not protect against killing induced by pCI mFasL transfection (293 cells)
Exp 1 - Transfect with 5 μg RID
- wait 24 h
- Transfect with 5 μg mFasL

Exp 2 - Transfect with 5 μg RID
- wait 24 h
- split the cells
- wait 24 h
- transfect with 0.5, 1, 2, 5 μg mFasL
- The MCF7 14.7 cell line (Terry) is not protected from killing with mFasL.
- I am trying to show functional RID in transfected cells (IP, IF).

EXHIBIT C17

- I am ~~trying~~ trying to show 14.7 in Terry's line (IF).
- I am expanding colonies isolated from 293 CrmA and MCF7 CrmA - mFasL transfections
- I am growing up virus (putative mFasL - β-Gal 7001) isolated from blue plaques.

Cancer therapy project
- 11/01/07 on WI 38 growing 10th day, arrested 7th day - no difference (no CPE). It seems that there might be a dill...

Mohan, March 7, 97

1. C.F. Project: AAV-ITR-KB/E1 growing C418 selected.

a) Preparation of plasmids pΔE1splA/SPC Rep78 and pBHG10 : under dialysis.

b) Construction of SPB-Rep78 plasmid - partial digestion + preparative scale -

c) RNA blot : For antisense Rep78 RNA in progress.

2. Cancer Project:

Experiment #3:

Growing HEL 299 cells 35^{th} day

a) dl 1101/1107 shows CPE in 100 & 500 pfu.

Growth arrested: 28^{th} day

a) pm 975 at 100 pfu shows CPE.

b) No CPE in dl 1101/1107 (100 & 500 pfu)

Growth Curve: dl1101/1107 (100 pfu) Total cell + Supernat

Growing: 5^{th} day = 1.4×10^4 pfu/ml

15^{th} day = 4×10^4 "

24^{th} day = 1.3×10^6 "

Growth arrested:

5^{th} day = 1.1×10^3 pfu/ml

10^{th} day = 1.4×10^3 "

19^{th} day = 8.4×10^3 "

23^{rd} day = 5.6×10^3 "

Do 309

Growth Curve: pm975 Growth arrested days (1 & 3) are titrated.

pm975 growth arrested Day 1 & 3. will be starting next week.

EXHIBIT C18

03-14-97 Kostya

I E3 region:

i) Initial stocks of the viruses with dlE3, ADP insert are prepared. Plaque assay on 293, A549 cells with 1101/1107, dl 309, ADP⁻ viruses as controls is to be started.

ii) Two large plasmids suitable for cotransfection with viral DNA based on Lynda's plasmid with E3 a) from dl 309 with gp19K mutated b) dlE3 X6a + ADP (-RID) are made. CsCl preps of these plasmids are done. Cotransfection of these plasmids with 1101/1107 DNA / EcoRI is in progress.
Will do one to TK gene, and PacI site.

II E4 - SP-B promoter:

i) There were 4 plaques which are developing CPE very slow in plate wells (Incomplete CPE in 3 weeks).

ii) Alternative project: E1 → SP-B (SP-C) promoter substitution. There was an article by David Solnick with E1 → MLP substitution (deletion E1 ~~promoter~~ -45 → +8 relative to E1 cap site).

However, I would propose to try to express E1A under the control of SP-B or SP-C in place of standard E1 deletion. Advantages: a) E1B would be completely deleted in the virus; b) It is possible to use 13S cDNA (express only 289 aa E1A)

EXHIBIT C19

Karoly 3/14/

Fas L project
- I did an IF with A549 and 293 cells transfected with pCDNA3 RIDα, pCDNARIDβ (alone and together and pCI RID. The results are inconclusive. I could see strong RIDβ expression where it was present, b the localization was mostly Golgi, even if RID was supposed to be there. (This was not easy to judge in 293. The IF did not work in A549).
The EGFR internalization did not work because of the antibody. I am going to repeat this experim in MCF-7.
- I have labeled ~~IP RIDβ, look for co-IP of RIDα~~ extract from the same transfection, I have not processed them yet.
- Fas L virus: I am doing a plaque assay with the isolated "blue" viruses.

Starting to test 293/Cre clones. Enough antibody? Transfect in Fas L - see if survives.

Conser therapy project
- I am doing an IF → DBP, BrdU incorporation with 1101/07 infected WI38
- Growing WI38 infected with 500 pfu/cell 1101/07 shows no real cpe on day 15
- Growing WI38 infected with 100 pfu/cell pm 75 shows cpe on day 3.

1101/1107 does not grow on primary cells. Perfect.

EXHIBIT
620

Gene Therapy Group Meeting 970314

Mohan, 3/14/97

1 C.F. Project:

a) pDE1splA/3.7 SPC Rep78 has one additional Xho1 site; Co-Transfected with pBHG10 in 293 cells.
~~Give to Tom - Western for E1A and E1B-19K.~~

b) KB MMT E1 Spinner cells, frozen some, MTV promoter ~~Regulated~~ & expanding =

c) RNA (293 Rep78) - ~~transferred~~ Blotted; synthesizing the RNA probe in progress.

2. Cancer project:

Growth Curve: pm 975 Growing:

total Cells Day 1 = 8×10^4 pfu/ml (New) Day 1 & 5 309 is now being titered.
plus supernatant " 3 = 7.1×10^7 "
" 5 = 1.3×10^7 " Day 6 - all
" 6 = 1.1×10^7 " all are floating.
" 7 = 7×10^6 "

Growing: Day 1, dl 1101/1107.
Day 3, dl 1101/1107)

Day 1, dl 309
Day 5 dl 309

These points are being titrated & stain Next week

Growth arrested:-
GA 1 DAY = dl 1101/1107

GA 1 Day = dl 309
GA 5 Day = dl 309

Need to call Bruce.

EXHIBIT

C21

3-21-97

Kostya

1) Sequencing of p21 (Nrippen's plasmid, EcoR1-B fragment 12-14 kb of pBHG11, E4 promoter deleted).

Primers directed to deletion point. Gal4 SP-C SP-B CMV

i) Ad5 sequence is OK ~ 600 bp downstream the deletion.

ii) Upstream the deletion Ad5 sequence 35770 → 35858 is as predicted. Upstream of Ad5 35858 nt there is sequence which is E. coli Tn5 according to BLAST. transposon 5. in ITR.

Probably, ITR is in plasmid but it can not be concluded from this sequence. Moreover, even if it is there, Tn5 is inserted ~77 bp from the right end of ITR making it not resquable to virus. Tn5 is at least 500bp & is prob much larger.

2) It is possible to cut out defective sequence from p82 (large plasmid with SP-B promoter) with Bst1107I and XbaI and put PCR product corresponding to Ad5 right ITR in single cloning.

left right end plasmid

Xba —— Bst1107I —— ITR – Xba

SP-B ← PCR

Nrippan - site in Gal4 promoter. Review Gal4 project.

EXHIBIT

C22

Karoly 3/3

Fast project
- I have transfected 12 293 CrmA clones and got partial protection in 7 of them. I will run a Western blot.
- I have tested 15 MCF7 CrmA-mFL clones in a Western blot (using α h Fas L). All seem to be negative. I will try mixing experiments.
- mFasL viruses: no blue plaque in 2nd plaque purification.

Cancer therapy project
- Growing WI 38 infected with 100 pfu/cell 1101/07: no CPE and
- Arrested WI 38 infected with 100 pfu/cell om 975: no CPE and
- Cell proliferation assay (BrdU incorporation => 17) shows that growing WI38 is not really growing.

EXHIBIT
C23

Mohan, 3/21/97

1 C.F. Project:

1st transfection was done last week

a) 2nd transfection was done in 293 cells; pBHG10 + pΔE1Sp1A/3.7 SPC Rep78

b) Northern Blot: for 293 Rep78 cell line, expressing Rep78 antisense RNA; blot is exposed to film.

c) checking E1 expression in KB MMTV E1 spinner cells Carried out induced by dexamethasone → Samples gave to Tudor Stark & BTR

~~Grow E1/E3 Cassette stocks.~~

2 Cancer Project:-

Growing 1 day: dl309 = 4×10^4 pfu/ml
5th day: dl309 = 4×10^6 pfu/ml

Growth arrested: 1 day dl309 = 1.4×10^5 pfu/ml
5th day dl309 = 1×10^6 pfu/ml

Growing day 1 = dl1101/07 = 4×10^4 pfu/ml

Growth arrested day 1 = dl 1101/07 = 1.2×10^3 pfu/ml

Buy spinners from MG

EXHIBIT
C24

Growth Curve. HEL 299 cells

Growing:		pm975	dl1101	dl309	pfu/ml all (100 pfu)
	Day 1	8×10^4	4×10^4	4×10^4	
	" 3	7.1×10^7			Repeat c̄ 500 PFU
	" 5	1.3×10^7	1.4×10^4	4×10^6	Do ~ 3-10d - to identify background
	" 6	1.1×10^7		—	Have CPE data c̄ 900 PFU
	" 7	7×10^6	—	—	
	10		—		Karl - next week c̄ WI38
	15		4×10^4		
	19		—		
	23		1.3×10^6		
	24		—		
Growth arrested	days 1		1.2×10^3	1.4×10^5	
	3				
	5		1.4×10^3	1×10^6	
	6				
	7				
	10	1.2×10^5	1.4×10^3		
	14	3.2×10^5			
	15	—			
	19	1.8×10^5	8.4×10^3		
	23		5.6×10^3		

Mohan, 4/4/97

1 C.F. Project:

a) 3rd Transfection was done; 3 transfections going simultaneously pBHG10 + pΔE1sp1a/3.7 SPC Rep78
Next week (18-20 days, should start seeing plaques. (From 1st transfection) On 293

b) Northern blot: 293 cells expressing antisense Rep78; RNA from 293 cells itself, gave hybridization signals (bands) with sense and antisense Rep78 RNA probe. May be DNA contamination? or unspecific bands?

293 cells transfected again c̄ Rep78 antisense (pCDNA3 Rep78); when the G418 resistant colonies forms, individual colonies ~10 will be propagated for the above RNA studies (or PCR).

c) checking expression of E1 in KB MMTV E1 spinner cells.

i) KB MMTV E1 cells induced with dexamethasone expressed E1B 19K; for E1a expression the antibody (- Tudor) is not good.

ii) I made a BHG E3 ITR LacZ virus prep from KB MMT E1 spinner cells (~1.5 liter); so E1 is functional in this KB MMTV E1 spinner cells.

iii) From 3 liter KB MMTV E1 spinner 5pfu/cell, 0.15 mM dexamethasone for 34 hrs gave viral band same as above

2. Cancer Project:

Experiment 3:- HEL 299 growing and growth arrested cells are infected with dl 110/wt; dl 309 (500 pfu/cell) = under progress for Growth Curve experiment

Need graph/table for trip to GTI.

EXHIBIT C26

Fas L project

- I did a Western with the 293 CrmA clones, they all express CrmA. I will cotransfect them to make FasL virus.
- Use this cell line to make Ad-FasL vector, perhaps Ad-14.7K and Ad-RID vectors.
- transfect FasL into cells. 1st expt - got protection.

E3 proteins

- I have isolated plaques from CMV RID and CMV 14.7 transfections and N RID and N 14.7 transfections.
  Nippon RID
  PGK promoter, R→L orientation
- Expression of RIDα from the CMV RID construct is ~~that~~ not proven yet.

Cancer therapy

- I am titering 1101/1107 yields on WI38
- There is some CPE in gm975 (100 pfu) inf. arrested WI38 on day 15

Make Ad vector (gene therapy) that expresses CrmA.
Use for gene therapy to keep transduced cells alive.

Lynda's expression cassette
all promoter, all Ad E3 genes. 1st 293, then MCF-7.
Do IP

4/11/97 Kostya

1) Cotransfection of 1101/1107 / EcoRI + p101 (dl 309 E3, gp19K mutated) or p111 (dlE3 X6a + ADP): There are 1 plaque with 101 and 1 plaque with 111 and no plaques in control dishes. Now amplifying these plaques to analize the genomes.

2) Need TK HSV-1 gene from GTI (if possible, with sequence and plasmid map). I'm going to insert the TK gene in PacI site of p101 (in gp19K gene) and X6aI site of p111 (~~before~~ upstream from ADP insert).

3) Failed once to replace Tn5 sequences with ITR in p82 (dlE4 promoter, SP-B promoter insert), now repeating the cloning.

1

Mohan 4/11/97

Gene therapy Meeting

1. C.F. Project:

a) Three different transfections were done to ~~make~~ construct SPB Rep78 virus. No plaques yet in one transfection (24 days)

b) dishes showed CPE like? in ~ 16 days in transfection; plaque titrations were done. Are started 4-11-97

c) 293 Rep78, antisense cell line were brought from the freezer; going to repeat the above transfections in Rep78 As. cell line.

d) primers are ordered for checking AAV Rep78 in 293 cell line.

2 Cancer project: HEL 299 cells, 500 pfu.

- Growing cells showed good CPE at ~ 15 days (dl 101/07)
- Growth arrested cells ~ 11th days very little CPE?
- dishes are being frozen for growth curve data

3. KB MMTVE1:-

pBabe puro plasmid is under preparation for Co-Transfection with E1 plasmid; pCMV plasmids

EXHIBIT

C29

Have pol II-ep14K, Cell stock, titer 1.4×10^7 | Karoly 4/11/87

Do IP ↑ this vector, compare to Ad5.

E3 proteins

Need to repeat

- Tested two VPGK 14.7 viruses for β-Gal - one expresses.
- Tested 3 CMV 14.7 and 4 CMV RID viruses for β Gal - none of them expresses.
- I have tried to sequence the CMV-RID construct, did not work. Lynda - point mutations in entire E3 region of Ad5 Need to do this, CMV promoter.

FasL project

- I have a ~~[illegible]~~ mixing experiment running with MC7~~7~~ - LumA - FasL cells, so far it is a maybe.

Cancer therapy

Preliminary titers:

Growing WI38 ~~[illegible]~~ infected with 500 pfu/cell 1101/1107

D ~~5~~ 1 < 10^5
D3 ≈ 1×10^5
D7 ≈ 3×10^5
D10 ≈ 2×10^5
D15 < 10^5
D20 ≈ 8×10^5
D25 ≈ 1.5×10^6
D30 ≈ 5×10^5

Quiescent WI38 infected with 500 pfu/cell 1101/1107

D 15 < 10^5
D 20 < 10^5
D 30 < 10^5
} no plaques at 10^{-5} dilution.

Will have ~~[illegible]~~ data on WI38 next week.

EXHIBIT C30

4/18/97 Kostya

1) Plaques, resulting from p101 (gp 19K mutated) and p111 (X6e dl(E3 + ADP): I have Hirt preps of genomes of these viruses, now analyzing by PCR and restriction digestion.

2) I have got clones with Tn5 → ITR substitution. Going to analyze them.

3) Starting to construct plasmids with Ad5, pm 760 RID genes under the control of CMV promoter. Same with the 14.7 gene.

Kostya will put E3 transcription unit, containing the pm 734.1 ADP mutations, behind the CMV promoter, for the purpose of inserting into E1 region of BHG11 (or 10).

EXHIBIT

C31

Xiaoly 4/10/97

E3 proteins

- I am going to test more NPGK 14.7 and NPGK RID viruses. (I am infecting today.)
- I did an IP with NPGK gp19k virus, no band on ON exposure.
- I have got the oligos for sequencing the CMV RID construct, I will do it this weekend.

Fas L project

- I am growing up 293 cells and making a CsCl stock of the plasmid (CMV m FasL in left end plasmid)

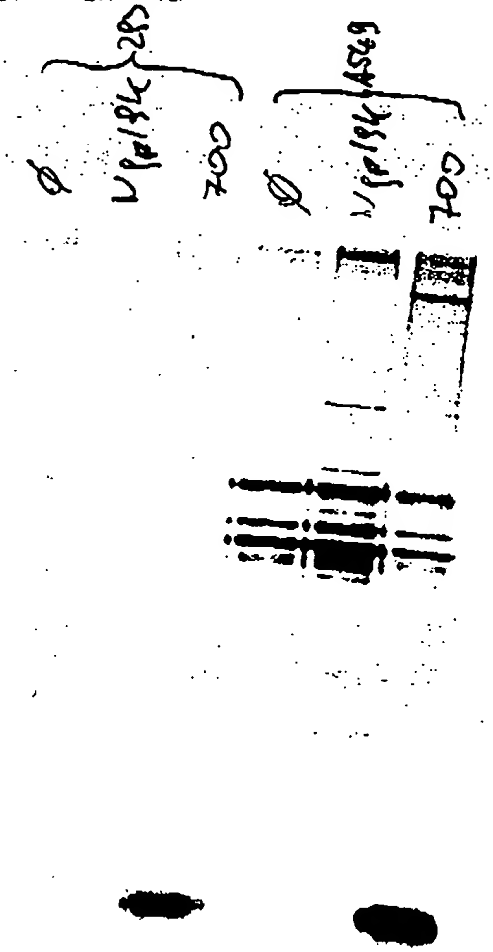
Cancer therapy project

Preliminary titers WI-38

	1101/1107 (500 PFU/cell) A	G	975 (500 PFU/cell) A	G	309 (100 PFU/cell) A	G
D1		1×10^5	1×10^5		3×10^6	
D3		5×10^5				
D5			1.5×10^5		1×10^7	
D7		3×10^5				3×10^7
D10		4×10^5	1×10^6			
D15	$< 10^5$	$< 10^5$	2×10^5			
D20	$< 10^5$	8×10^5	8×10^5			
D25		2.3×10^6	7×10^5			
D30	$< 10^5$	7×10^5				

EXHIBIT
C32

4/18



Could see by IF, beginning at 2 days pi

EXHIBIT
C33

Mohan, 4/18/97

1. C.F. Project:

a) No plaques in 293 cells co-transfected with pBHG10 and pΔE1sp1a/3.7 SPC Rep78. (~ 30 days and 24 days); 3rd transfections (18th days) dishes are stained; waiting for ~~plaques~~ plaques.

b) Cells showed CPE like effect (pBHG10 + pΔE1/SPC Rep78) were titrated and not stained yet. But even undiluted dish did not show CPE.

c) RT-PCR showed no Rep78 DNA fragment in 293 and 293 ∠Rep78 A.S. cell lines.

d) Spinner KB MMTV E1 cells → virus IR LacZ is ~~[illegible]~~ in spinner; Karl virus is going next.

e) pBabe puro plasmid is made in large quantities; for transfecting the KB E1 cell lines with E1 and CrmA plasmid separately.

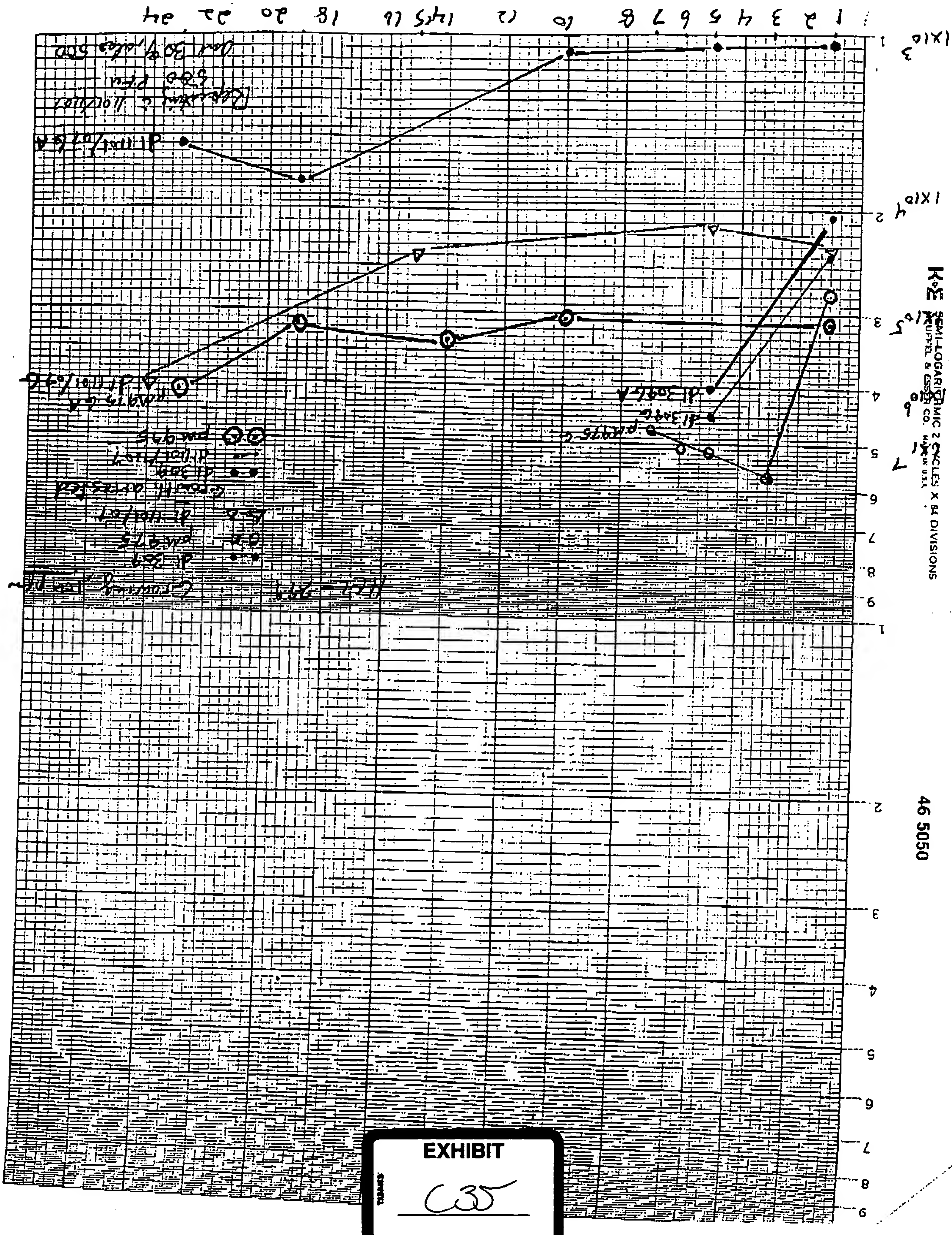
2. Cancer project :- HEL 299 cells; 500 pfu
- Growing cells were dead at ~ 21 days.
- Growth arrested cells 18th day. On 21st day cells will be frozen for Growth Curve analysis.

3. preparations: for transfection: plasmid BHG10, BHG E3, pΔE1/SPC Rep78; will be CsCl purified.

293 ∠Rep78 AS cell line will be co-transfected c̄ BHG10, BHG E3, ARE DNA + pΔE1 Sp1A/3.7 SPC Rep78 ~~plas~~ plasmids

EXHIBIT C34

FROM :SAINT LOUIS UNIVERSITY MMI   FAX NO. :3147733403   Dec. 19 2002 12:32PM P8



EXHIBIT

C35

4/25/97 Kostya

1) I have got the virus 1101/1107 with gp19k mutated (restriction digestions and PCR).
2) Now preparing Hirt's from 4 plaques which should be 1101/1107 E1; X6a E3 deletion, ADP insert
3) Failed to substitute Tn5 → ITR. Going to do it in 2 clonings but without partial digestions.
4) Sequencing shown that ITR is OK in Lynda's plasmid, could not sequence through ITR-ITR junction in pBHG10, 11; I have repeated the sequencing with different PCR protocol, now waiting for results.
5) I have checked with Ann, she doesn't have plasmids with rec 700 or pm 734.1 which would be suitable to cut out SrfI - NdeI fragment corresponding to whole E3, but she does have the viruses. Now going to prepare rec 700 and pm 734.1 DNA to insert SrfI - NdeI fragment to pCDNA3 / EcoRV site.
6) The virus 544 (1101/1107 E1, Lynda's deletion of E3 ~~[illegible]~~, 12.5 K ATG mutated, dl 27984-28133 dl(28390-30883) + ADP insert into PacI site in place of second deletion. ⇒ I gave the virus to Shari to grow up CsCl prep.

Need TK gene from GTI.

EXHIBIT
C36

Karoly 4/25/97

E3 proteins

- I have tested more NPGK 14.7 and RID viruses. The ones that stain for β-Gal show some staining in IF for 14.7 or 14.5. The RID β staining shows the same pattern as the previously made ~~Ad~~ Ad RID viruses. In addition to this, the cells that shine are dead (carryover from 293?). The 14.7 staining looks normal. I am growing up more of the positive clones.

- FasL project

- I have two transfections going in 293 CrmA.

Cancer therapy project

- I have infected MCF-7 with 01/07, 975 and 309. Will do the same with A549 and 293 and take a growth curve.

- I am infecting WI38 for the missing timepoints.

- I am titering the remaining samples.

	1101/1107		175		309	
	A	G	A	G	A	G
D1		1×10^5	1×10^5	4×10^5	3×10^6	$< 10^5$
D3		5×10^5				2×10^7
D5			1.5×10^5	3×10^5	1×10^7	4×10^7
D7		3×10^5				
D10		4×10^5	1×10^6	1.4×10^7	6×10^7	
D15	$< 10^5$	$< 10^5$	2×10^5		2×10^7	
D20	2×10^3	8×10^5	8×10^5	1×10^6		
D25		2.3×10^6	7×10^5			
D30	$< 10^5$	7×10^5		1×10^6		

She 11 Chart 2

Approximate titers in WI38 4/25/97

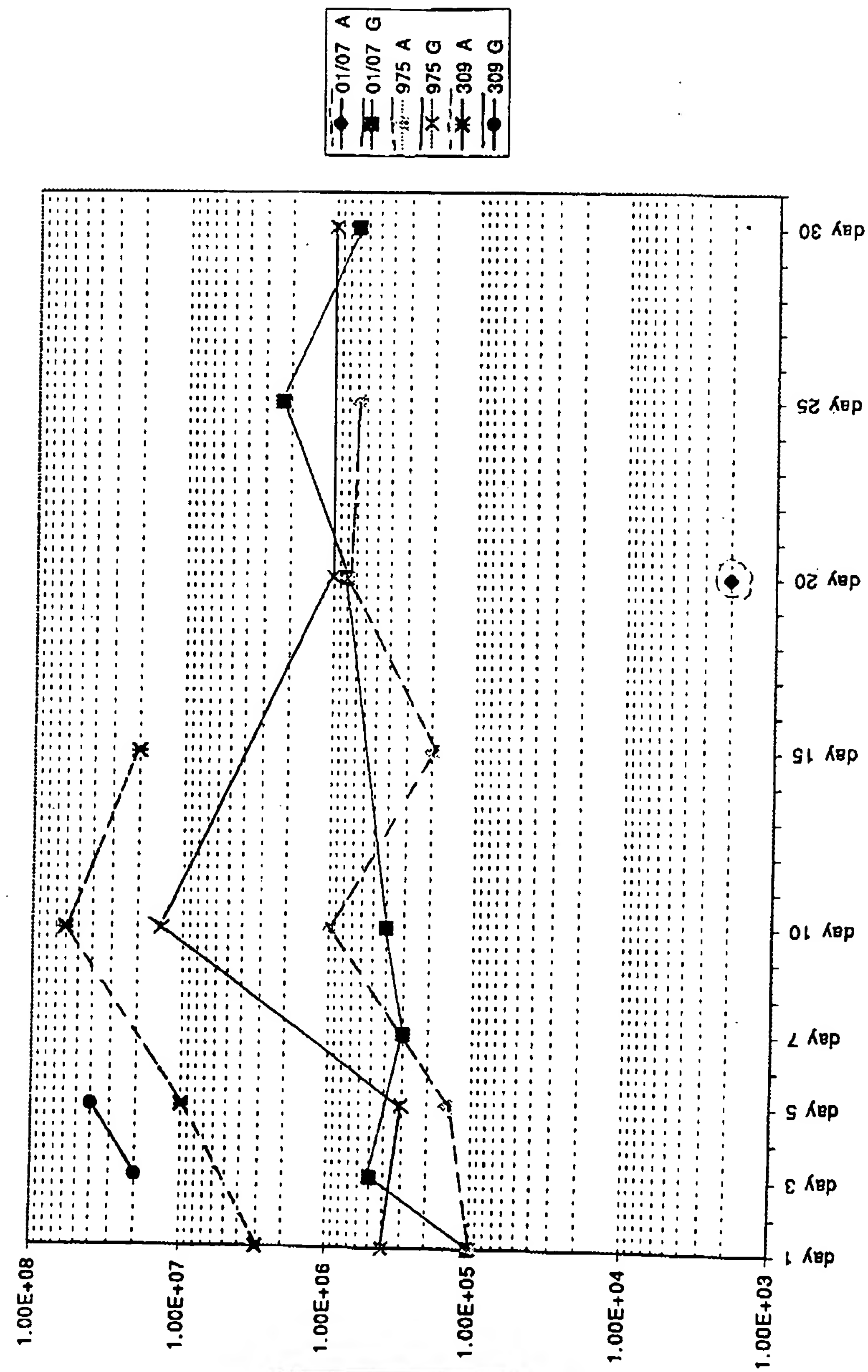


EXHIBIT
C39

Mohan, 4/25/97

1 C.F. Project:

a) cells showed CPE like effect (co-transfected pΔE1sp1A/3.7 SPC Rep78 and pBHG10) were titrated. No plaques yet ~15th day. But when 293 cells infected with CPE, CPE like started look Ad CPE typical.

b) In 293 Rep78 AS - cell line, co-transfections of pΔE1sp1A/3.7 SPC Rep 78 and pBHG10 were done; pΔE1sp1A/3.7 SPC Rep78 and pBHGE3 plasmids.

c) Virus Ct 19.7 are growing in KB MNJV E1 Spinn calls.

d) GAPDH (Glyceraldehyde 3Phosphate dehydrogenase) primers - ordered.

e) pBHG10, pBHGE3 under preparation for further transfections.

f) I am going to isolate dl7001 DNA for transfection → virus Rep78.

2 Cancer Project: HEL 299 cells

- Growing and Growth arrested cells are frozen for growth curve analysis.

9/2/97 Kostya

1) Terry's gp19K plasmids. pCR11gp19K Ad5 turned out to be pCR11gp19K Ad2. pCIgp19K contains gp19K Ad2 in correct orientation. (Karol - Transfection - IP).

2) Cloning of the complete E3 under CMV promoter;
i) There was E3 intron in Horwitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
ii) Started to extract rec 700 and pm 734.1 (m1m41) genomes to clone E3 to pCDNA3 (or pCI?).

3) Cloning experiments to replace ITR in SP-B⇔E4 promoter plasmid are in progress.

4) Got the virus 1101/1107 E1; dl(E3 X6a + ADP — grows much better than dl7001 — checked with restriction digestion and PCR. Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + ADP; dl309 gp19K mutated; dl(E3 X6a + ADP). Using dl309; 1101/1107; pm 734.1 (m1m41); 7001 as controls. Cell lines - 293 and A549.
Going to start with IP to check for ADP expression
I'm really ready to put TK into 2 last recombinants E3, need TK plasmid and TK sequence from GTI.

Need TK from GTI!

Karoly 5/2/97

E3 proteins

lacZ pGK-14.7k Nolan got smaller band.

- I am growing up NPGK 14.7 #5 showed best IF.
- I did an IP with transfected pp 13k and 14.7 constructs (in 293 cells). All but pCDNA3 14.7 expressed, all comparable to virus injection. (Labeling 38 h post TF and 18 h p.i. for 3h)
- I did an IF with the same transfections (in MCF7 Fas), it did not work, I am repeating it.

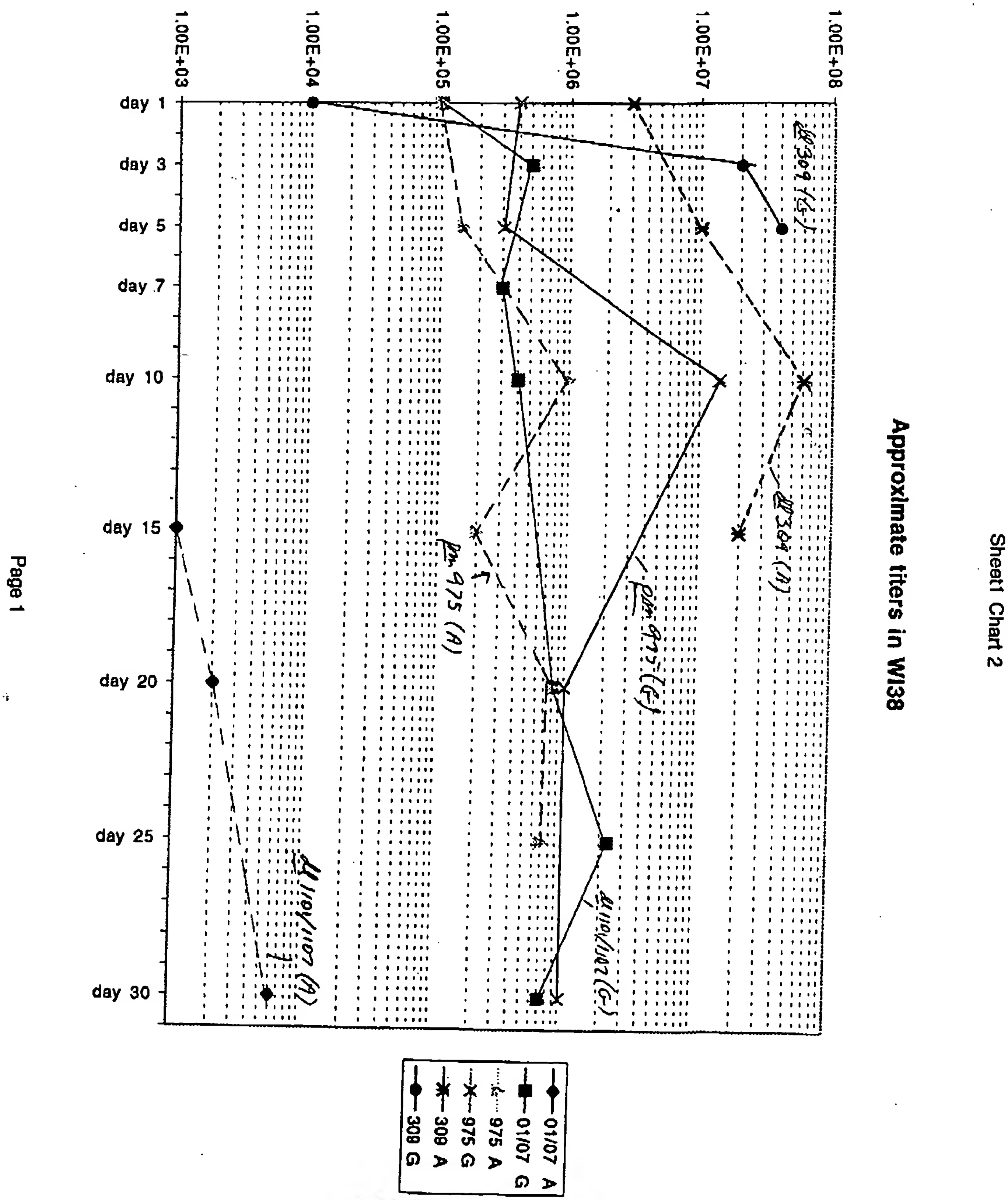
FasL project

- I am waiting for plaques in 293-Crm cells.
Have one plaque after 10 days.

Cancer therapy project

		01/07	975	309
I have infected	MCF7	500 pfu	100 pfu	100 pfu
	A549	500 pfu	500 pfu	500 pfu
	293	500 pfu	100 pfu	100 pfu

293 is rounded up 1 day p.i.
A549 2 days p.i.
MCF7 5 days p.i.


Sheet1 Chart 2
Approximate titers in WI38
1.00E+03
1.00E+04
1.00E+05
1.00E+06
1.00E+07
1.00E+08
day 1
day 3
day 5
day 7
day 10
day 15
day 20
day 25
day 30
dl309 (G-)
dl309 (A)
pm 975 (G-)
pm 975 (A)
dl1101/1107 (G-)
dl1101/1107 (A)
01/07 A
01/07 G
975 A
975 G
309 A
309 G
Page 1

EXHIBIT

C43

9/2/97 Kostya

1) Terry's gp19K plasmids. pCRII gp19K Ad5 turned out to be pCRII gp19K Ad2. pCIgp19K contains gp19K Ad2 in correct orientation. (Karol – Transfection – IP).

2) Cloning of the complete E3 under CMV promoter;
i) There was E3 intron in Horwitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
ii) Started to extract rec 700 and pm 734.1 (m1m41), genomes to clone E3 to pCDNA3 (or pCI?).

3) Cloning experiments to replace ITR in SP-B⇔E4 promoter plasmid are in progress.

grows much better than dl7001

4) Got the virus 1101/1107 E1, dl(E3 X6a + ADP) – Checked with restriction digestion and PCR.
Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + ADP; dl 309 gp19K mutated; dl(E3 X6a + ADP). Using dl 309; 1101/1107; pm 734.1 (m1m41); 7001 as controls. Cell lines – 293 and A549.
Going to start with IP to check for ADP expression
I'm really ready to put TK into 2 last recombinants E3, need TK plasmid and TK sequence from GTI.

Need TK from GTI!

EXHIBIT
C44

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

Mohan, 2/21/??

1. C.F. Project:

a) BHGE3 ITR LacZ virus gave moderate band in CsCl banding. But the β gal activity/ml is ~1.4 x10^{9} which is same as previous ITR LacZ preparation. Second set is being titrated to compare the pfu/Lfu/ml.

I may use Centricon 100 (M.wt cut off 100,000) to concentrate the viral stocks for one CsCl spinning which will increase the pfu.
Dialysis of stock ITR LacZ virus will increase the volume and it is hard to spin in one tube.

b) Co-transfection of pEL SpIA/3.7 SRC Rep78 & pBHG10; pBHGE3 in 293 Rep78 antisense cell line will be stained ~~next~~ week

2. E3 project:-

a) Virus Ct 14.7 (pGK II 14.7 + β gluc) gave moderate band in CsCl spinning.
Viral tirations are in progress.

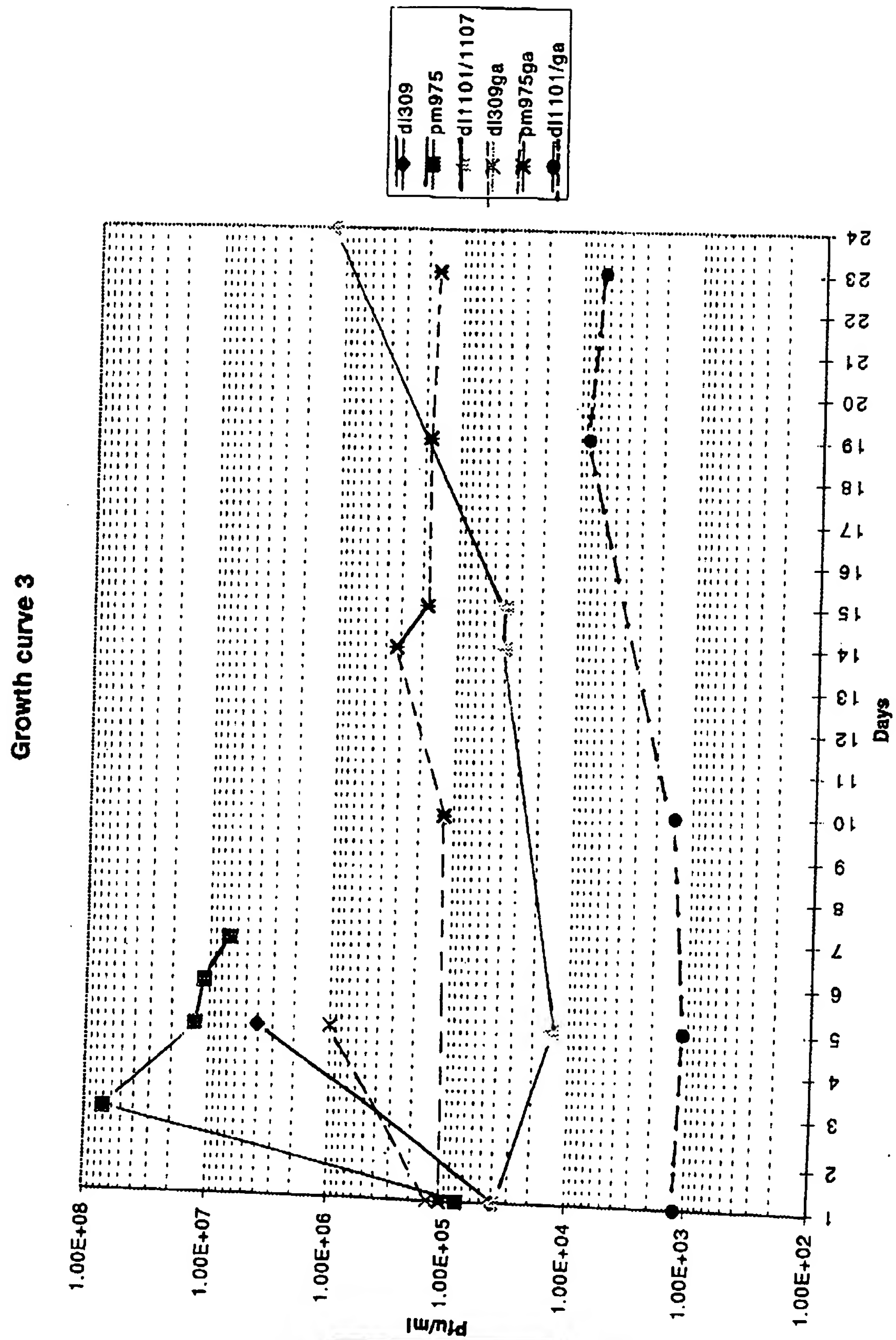
b) Going to make another virus 14.7 LacZ (pGK 14.7 in E3; LacZ in E1) preparation.
- infection today

3. Cancer Project:-

Growing and growth arrested virus infected HEL 299 cells ~~[illegible]~~ extract will be titrated for growth curve next week.

dl 1101/1107 at 500 PFU/cell
dl 309 " " "

Sheet1 Chart 3



EXHIBIT

C46

05-01-91

Kostya

1 i) Results of cotransfection 1101/1107 /EcoR1 + p54 (dlE3 + ADP) → 4 more plaques were analyzed. 3 of them contain recombinant genomes.

ii) Subcloning of dl 309 E3 with gp19K mutated and dlE3 Xba + ADP into Linda's plasmid is in progress. When the plasmid with 19K * is made I'm going to insert HSV-1 TK into PacI site created in gp19K gene.

2) i) No plaques with p628 (pBHG11 E4 promoter → CMV promoter). No plaques with pBHG11 as a control.

ii) 4 more plaques resulting 1101/1107 /EcoR1 + p82 (E4 → SP-B promoter) were analyzed. All contain wt genomes.

iii) In 2 repetitions of cotransfection 1101/1107 with p82 (E4 → SP-B promoter) no plaques neither in control no in experiment. 2nd experiment was done on Mohan's 293/CMV-TTF cells.

Now repeating the cotransfection.

iv) Sequencing of Nripen's plasmid p21 (E4 promoter dl) is in progress to ~~prove~~ prove that right Ad ITR is intact in the plasmid.

3) Growing initial stocks of the recombinant ~~virus~~ virus with dlE3 + ADP.

EXHIBIT

C47

Gene Therapy Group. Karoly 5/9/97

E3 proteins

- I am growing up NPGK 14.7 #5
- I did an IF with transfected gp19k and 14.7 constructs in MCF 7 cells, all of them but pCDNA3 14.7 expressed well.

Viruses - NPGK gp19k and βGluc 14.7 were negative after 20h.
Mohan is doing a longer infection assay

Fast project

- I have 4 plaques. They appeared Saw plaques at → 10-11 days after transfection with BHG11 in 293 Cre A cells.
Will pick one plaque (large) today.

Cancer therapy project.

293, MCF7, last time mistake: Modified
Repeat c A549.

I am repeating A549 infections and some WI 38 infections.

CPE in WI 38 cells

A = 0.2% serum FCS
G = 5% FCS

01/07 A
no CPE

01/07 G
"atypical CPE" starting around day 10

No CPE c 1101/1107, either in growing or nongrowing
But, have fewer cells in the growing than non-growing

500 pfu

The "growing" don't really grow!

975 G
D2 +/-
D3 +/-
D4 +
D5 ++
D6 +++

No real diff. between G and A for pm975
500 PFU

975 A
D4 +/-
D5 +
D6 ++
D7 +++

500 pfu

For 100 PFU, growing was same as 500 PFU
But, arrested did not show CPE until ~15 days. Same c HEL-299.

308 G
D3 +/-
D4 +
D5 ++
D6 ++

308 A
D5 +
D6 +
D7 +

100 pfu also pCE

Bring pCDNA3.1. Clone gp19, 14.7, RID
pMT2-14.7 pMT2-gp19K.

...e photograph
...as done quickly
...0 PFU.
Next Week
...plaques
A-CAT to test for
free EGF
...38, HEL-299
...ming resting
...ll cell types.

5/9/97 Kostya

1) Construction of the plasmids with whole E3 (rec 700, pm 734.1) is in progress.

2) I have constructed the plasmid with E4⇔SP-B where Tn5 ⇔ ITR, sequenced the plasmid, now waiting for sequencing results to make the ~~large~~ version of the plasmid for cotransfection.

3) For 101-1 (dl 3.4 E3, gp 14K mutated) plaques are appearing the same day as for 309 (day 4). For 111-1 (E3 Xba dl + ADP) plaques appeared 1 day before dl 309. (day 3)

① KD-1. 1101/1107 in Lynda's E3 background + ADP
No promoter for ADP.
Shari harvested day 2 pi, got band, will titer.
Cells were beginning to die. Shari will titer.

② KD-2 1101/1107 → 309 E3, gp19K mutated.
Plaques appearing same day as 309.

③ KD-3 1101/1107, XbaI deletion (dl 327 deletion), ADP without promoter, but in-frame c 6.7K.
Got plaques 1 day before dl309 (ie on day 3).
Got this one plaque 4 days posttransfection, 5 mm wide.
Got other 3 plaques 7 days posttransfect
DNA screened - are recombinant

Future - could put ADP under control of SPB promoter.
First, concentrate on what we have.

EXHIBIT
C49

~~infect~~ pAT2-ADP with 29.3 ....

Mohan, 5/9/97

1. C.F. Project:

a) Virus BMG E3 ITR LacZ gave 1.4×10^9 Lfu/ml ~~Repl assay~~
Plaque assay gave 9×10^8 pfu/ml (10th day)

I received Centricon 50 concentrator from Millipore as Samples - going to concentrate the ITR LacZ virus

b) So far no plaques for constructing Rep78 virus.

c) GAPDH - RT-PCR in progress.
d) Going to transfect dl 7001 DNA ClaI digested, with pSPC Rep78 plasmid.

2. E3 project:
~~Positional CMV enhancer, left-to-right orientation~~

a) Virus C+ 14.7 (pol II 14.7 + β gluc) has been Titrated. No plaques yet - 10th day.
b) CPE stock for 14.7 LacZ (pol II 14.7 + LacZ) virus has been made. Going to titrate. (5ml final)
c) A549 cells are infected c̄ 1700, GP19k and C+ 14.7 virus for I.F. (2 days & 4 days).
Read 2 days - ~~negative~~ already infected.

3. Cancer project:- HEL 299 cells
Growing, growth arrested, dl 1101/07 (50 pfu) infected - CPE stocks are Titrated for growth curve
Will do 309 in coming week

HEL-299 100, 500 PFU Graph: days pi vs CPE - Will give me.

EXHIBIT
C50

5/23/97 Kostya

1) E3 project:

Cloning of E3 from rec 700 and pm 734.1 in pBSSK (+) - got bacterial colonies, analyzing, then reclone into pCL, then make virus.

2) Cancer therapy project:

i) Made a large plasmid (Bam → end of genome) with E4 → SPB promoter and ITR repaired, now making CsCl prep. of the plasmid to transfect. Next week make virus.

ii) Plaque development assay for E3 ADP viruses:

a) 293 cells (day 18)

Negative control:

7001 — plaques just started to appear (day 18).

734.1 — plaques appeared day 14-15

Positive control:

309 — day 4-5

1101/1107 - day 4-5

Recombinant viruses:

KD1 (544, LKH dl E3 + ADP) — day 5-6, plaques smaller than wt.

KD2 (101-1, dl309, SP 19K *) — day 4-5, large plaques.

KD3 (111-1, dl 327 + ADP) — day 4-5, large plaques. Same day a little faster than WT Perhaps a little larger.

b) A549 cells (day 8)

Positive control: 309 - day 4-5 (large)

1101/1107 — day 8 (small)

Negative control: 734.1; 7001 - no plaques yet.

Recs. KD1 - didn't make

KD2 — day 5-6, large, smaller than wt (309)

KD3 — day 5-6, smaller than KD2.

Mohan, 5/23/97

1. C.F Project:

a) virus BHGE3ITR LacZ was concentrated using Centricon filter columns. Lfu is ~ 3.86 x 10^10/ml [cl/ml]

b) dl 7001 viral DNA digested with ClaI and pΔE1sp1A/spcRep[78] were co-transfected

c) 293 TTF cell lines were transfected with pΔE1/spcRep[78]. I.F stained for Rep78. Most of cells transfected with pΔE1/spcRep78 are washed out. Few cells (about 2) showed nuclear staining. But not clear.

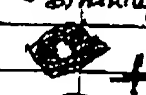
d) 293 ∠Rep78 antisense cell lines showed GAPDH-PCR fragment not the Rep78 fragment. So, no AAV2 contamination.

2. E3 Project:

a) NPGK LacZ 14.7 (CPE stock) gave = 3 x 10^8 Lfu/ml in 293 cell but in A549 = 2.3 x 10^9 Lfu/ml, ~ 10 fold more in A549 cells than 293 cells

b) I.F = A549 cells: ~ 50 pfu/cell

r 7p0 —	21 hrs (21 hrs) ++++	for 14.7
NPGK 14.7 —	1 day	—
	2 day	—
(Boston P/c fluorescence)	4 day	++++
Ct 14.7	~ 4 day	++ (cytoplasmic staining)
GP19K	= Background	



Virus NPGK 14.7 expresses 14.7 ~ 4 days post infection at wt. level.

Ct 14.7, at 4th day expresses lesser. Mainly cytoplasmic staining and around the nucleus.

Ct 14.7 when titrated - 10^{-7}, 10^{-8}, 10^{-9} no plaques. — Titer may be less. So, -IF- may be less.

EXHIBIT

C52

3 Cancer project: HEL 299 cells 500 pfu/cell

Growing

	dl309	dl1101/07
3hrs	9×10^3	2×10^4
1 day	1×10^4	4.2×10^4
4 "	1.6×10^7	1.2×10^5
6 "	2.3×10^7	—
8 "	9×10^6	1.4×10^5
10 "		7.1×10^5
15 "		2.2×10^6
21 "		4.6×10^6

Growth arrested

3 hrs	$\sim4\times10^4$
1 day	4.4×10^4
4 "	4×10^4
8 "	5×10^4
10 "	$\sim1\times10^4$
15 "	$\sim1\times10^4$
21	$.2\times10^4$

EXHIBIT C53

Karoly 5/23/97

E3 proteins

- I am doing an IP with NPGK 14.7 and Ct 14.7
- I am growing up lac-Pol II gp19K
- I am cloning the MT2 expression casette in pC12PacI (Hyuda changed the BamH1 and BglII sites to PacI in pC1)

22-141 50 SHEETS 22-142 100 SHEETS 22-144 200 SHEETS AMPAD

Fas L project

Could be WT.

- I have isolated 4 plaques. They grow very fast (kill 293 ON, show CPE in A549 ON).

  I have infected MCF7 and MCF7crmA cells, it shows CPE ON in both.
- I have tried to PCR out Fas L from the virus, it failed. Positive controll (plasmid) was OK.
- The virus expresses β-Gal
- I am trying to show the protein in IF.

Cancer therapy project

- I am going to titer yields from A549, MCF-7 and 293 next week.

EXHIBIT

C54

5/30/97 Kostya

1) E3 project;

Made the plasmid with E3 from pm 759.1 (m1/m41). (ADP⁻) - Sfi - NdeI - fragment in pBlueScript. Now cloning is in progress to put it into pCDNA3.1Zeo(+) - new plasmid with CMV promoter. Now just started

2) Cancer therapy project;

i) Made cotransfection of 1101/1107 / EcoRI with p152 (Bam→mp100 ⇌ SPB in E4) (new version of large plasmid Bam - [illegible] with SPB ⇔ E4 promoter substitution). to 293 and promote 293-TTF cells, cotransfected pCMV/TTF as well. Has ITR (made by Mohan)

ii) Plaque development assay for E3 ADP viruses:

293 cells - see graph.

A549 cells - in progress.

iii) Starting IP with Ab ADP sera to check for ADP expression in the viruses direct.

Kostyu

Plaque development assay, A549 cells

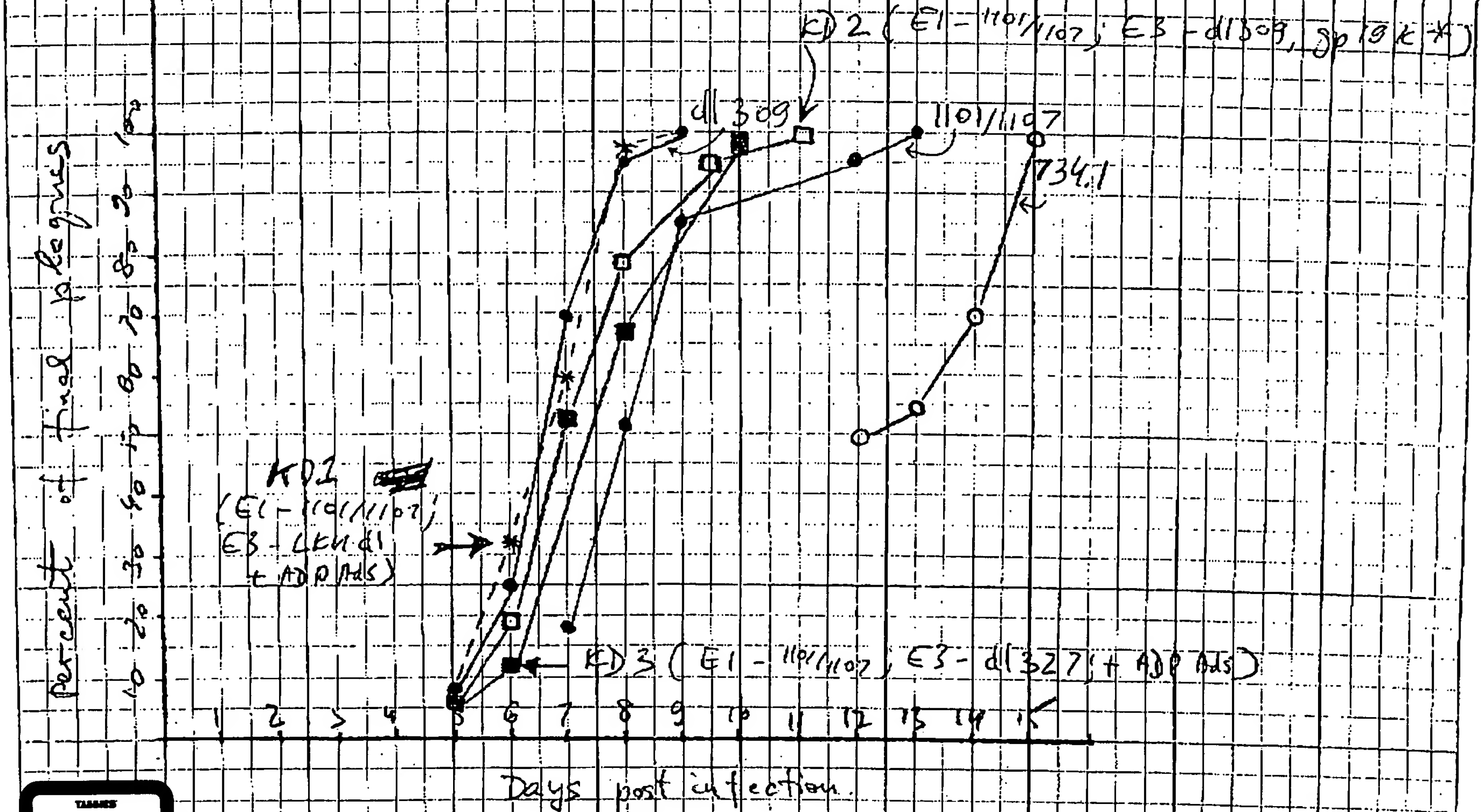


EXHIBIT
C56

Karoly 5/20/97

E3 proteins

- I did an IP with NPGK 14.7B, the result is not conclusive.
- I am repeating the MT2 clonings.

Fas L project

- The tested four plaques were not expressing β-Gal. I found two blue cells, I don't know where they came from.
- I have 3 new plaques, they came at the same time as lacZ controls.

Crm A

- I am cloning CMV-CrmA in the left end plasmid

Cancer therapy

- I am titering the time course in A549, MCF-7 and 293 cells! Started yesterday.

E2-Gal

I am waiting for plaques (24 and 8 days).
No plaques on lacZ control yet either.
In Chip's GAL4-TAD 293 cell line

BHG11, E2A promoter substituted c̄ Gal4/TATA.

Chip - give Ad2 × Ad5 combo. in pCI, to Karl. Make Ad vector ± lacZ
- give RSTLTR-14.7K, pCI-14.7K, pMT2-14.7K to Mohan. Made Ad vector ± lacZ
Ustya - will handle gp19K/CMV, Ad2 version.

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Dec. 19 2002 12:39PM P12

Plaque development assay (293 cells)

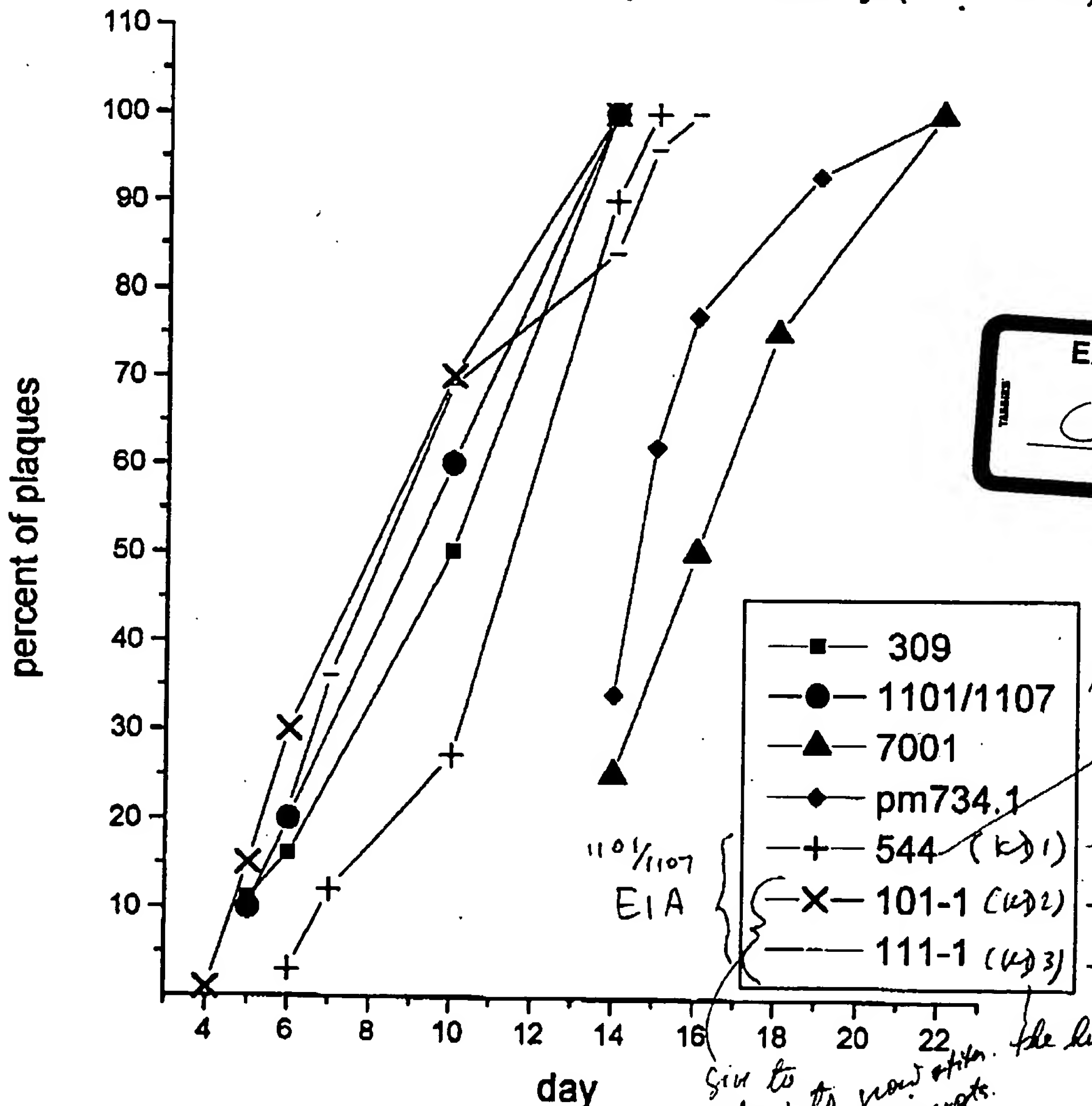


EXHIBIT
C58

little bigger plaque
Very strong CPE

– Lyndei's dl/ + ADP
– E3 309 gp 19 K
– dl E3 x6g + ADP

the huge plaque

He thinks get expression in early stages.

Give to Shari to grow stock.
Ann will do killing expts.

Make one 7 KD1,2,3
also looking E1B-19K.

A549 expt – now at 14 days. Look like 293, but 309 is a bit faster.
1101/1107 mutant does show clearance [illegible].

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Dec. 19 2002 12:39PM P13

Mohan 5/30/97

1. C.F. Project:

a) 293 cells (< Rep78), transfected by dl 7001 DNA (2 wk) started showing plaques (~40/60mm dish) ~15 days

- cells co-transfected with pΔE1 sp1A/3.7 SPC Rep78 and dl 7001 + ClaI digested viral DNA — no plaques yet. (15th day)
- cells transfected by dl 7001 + ClaI — no plaques —

(I am going to several transfections using dl7001 + claI digested DNA)

b) MCF-7 ~~cells~~ cells were transfected — as following — plasmids for I.F. for Rep78

1) pcDNA3 Rep78 (I.F will show Rep78 expression)
2) pCMV TTF
3) pCMV TTF + pΔE1 Sp1A/3.7 SPC Rep78
4) pΔE1 Sp1A/3.7 SPC

c) Primers for Rep78 antisense → ordered.

2. E3 project:

a) Viruses Ct 14.7 (βactin promoter), 14.7 LacZ (pol II 14.7)
did not show plaques at 10^{-7} dilution.

particles/OD; for wt Ad 9.04×10^{13}/ml — std.

		1	2		
		260	260	pfu	particle/OD → OPD
CsCl	r700	0.174	0.167	9.88×10^{10}	~1.54×10^{12}
	Ct 14.7	0.232	0.217	—	~1.9×10^{12}
CPE Stock	ΔPGK 14.7	0.869	0.861	Lfu 3×10^8 293, 2.3×10^9 A549	—

(Medium is interfering)

— Going to repeat with more dilutions.

b) After Ct 14.7 and 14.7 LacZ pfu → I.F.

EXHIBIT C59

3. Cancer project: HEL 299 cells; 500 pfu/cell

dl 309 growth arrested cells → stained

→ plaques start~~ed~~ appearing Count ~2 day

EXHIBIT

C60

Sheet1 Chart 6

Growth curve 500 pfu/cell

(HEL 299 cells)

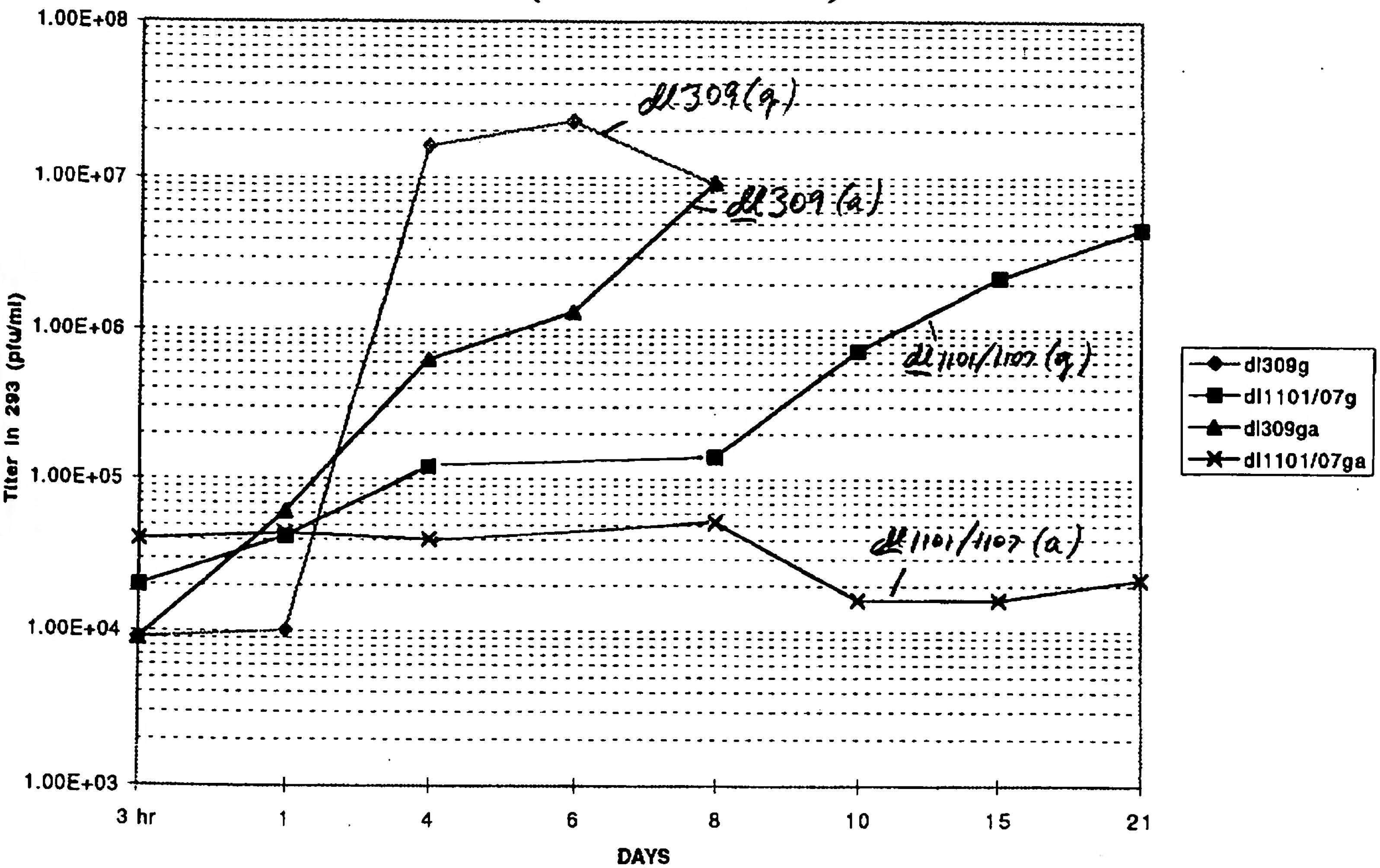


Figure 1.

Sh et1

Yields in A549 infected with 100 pfu/cell pm975 or dl309, or 500 pfu/cell dl1101/1107
6/9/97

days\viruses	1101/1107	pm975	dl309
1	7.00E+06	1.00E+07	1.00E+07
5	3.90E+07	3.00E+07	1.00E+08
10	4.00E+07	1.00E+08	4.00E+07
15	3.10E+07	3.00E+07	2.00E+07

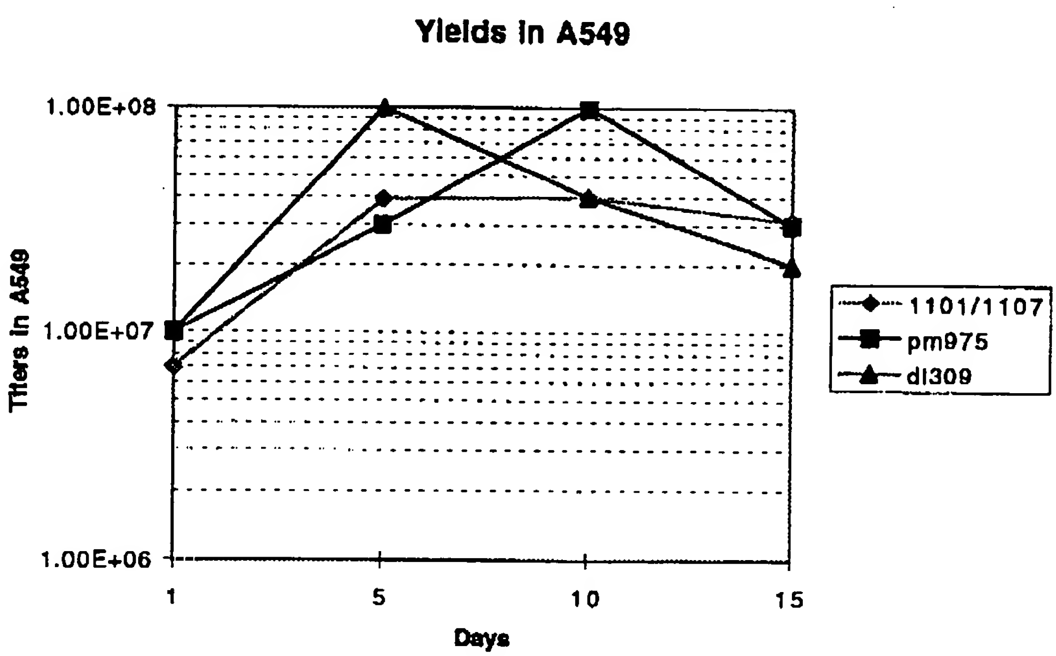


EXHIBIT
C62

Sheet1

Yields in MCF-7 infected with 100 pfu/cell pm975 or dl309, or 500 pfu/cell dl1101/1107
6/9/97

days\viruses	1101/1107	pm975	dl309
1	1.00E+05	1.00E+06	4.00E+06
5	1.00E+06	7.00E+06	
10	1.50E+06	8.00E+06	3.70E+07
15	1.00E+06	1.00E+07	1.00E+08

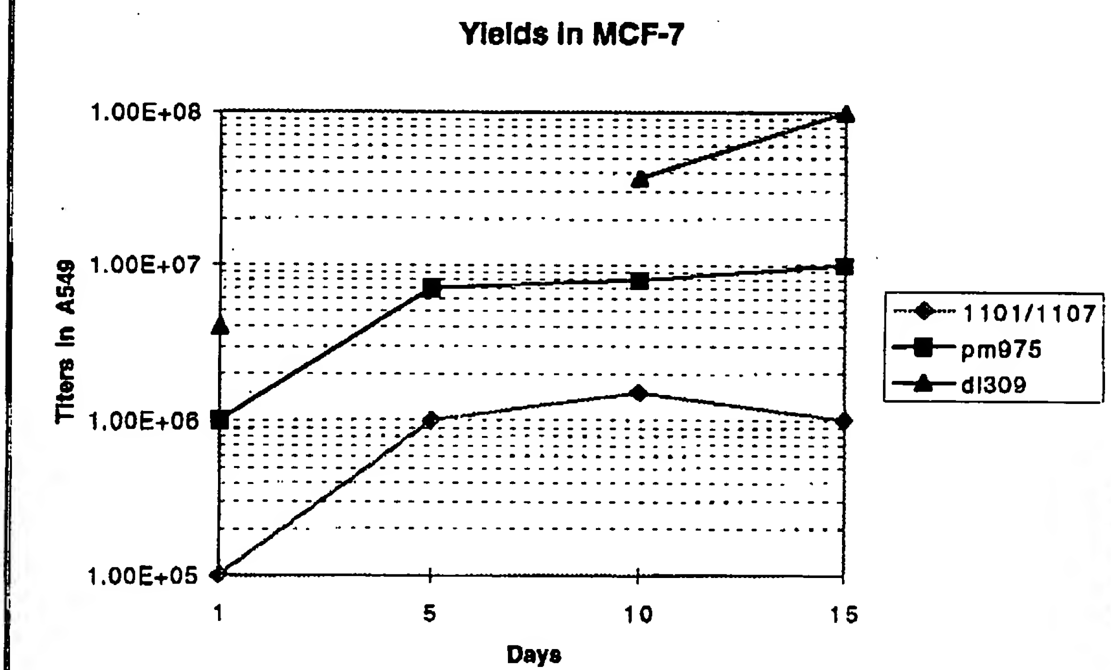


EXHIBIT
C63

Sheet1

Yields in 293 infected with 100 pfu/cell pm975 or dl309, or 500 pfu/c ll dl1101/1107
6/9/97

days\viruses	1101/1107	pm975	dl309
1	2.70E+07	1.00E+07	1.00E+08
5	2.60E+07	1.00E+07	9.00E+07
10	1.90E+07	1.00E+06	1.00E+08
15	6.00E+06	4.00E+06	2.00E+07

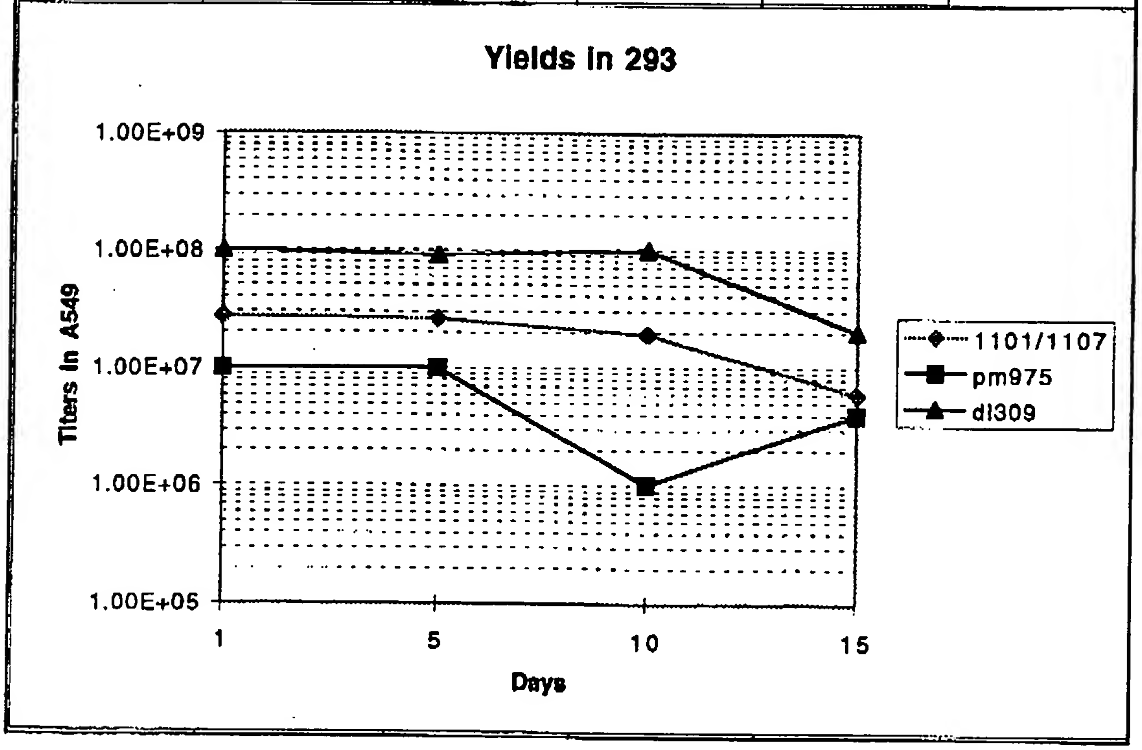


EXHIBIT
C64

6/13/97 ROS'y.

1) E3 project

Made the plasmid with E3 (SrfI - NdeI) from pm 734.1 (ADP⁻) in pCDNA3.1 zeo(+). Gave maxiprep to Karol to check for E3 proteins.

2) Cancer therapy project

i) There are no plaques yet in dishes with p152 (E4ΔSPB) transfection (2 weeks).

ii) Plaque development assay for E3 ADP viruses in A549 — see graph.

iii) IP with anti-ADP serum didn't work (even in positive control). Next week going to try IP with controls and various sera to optimize experiment conditions.

EXHIBIT

C65

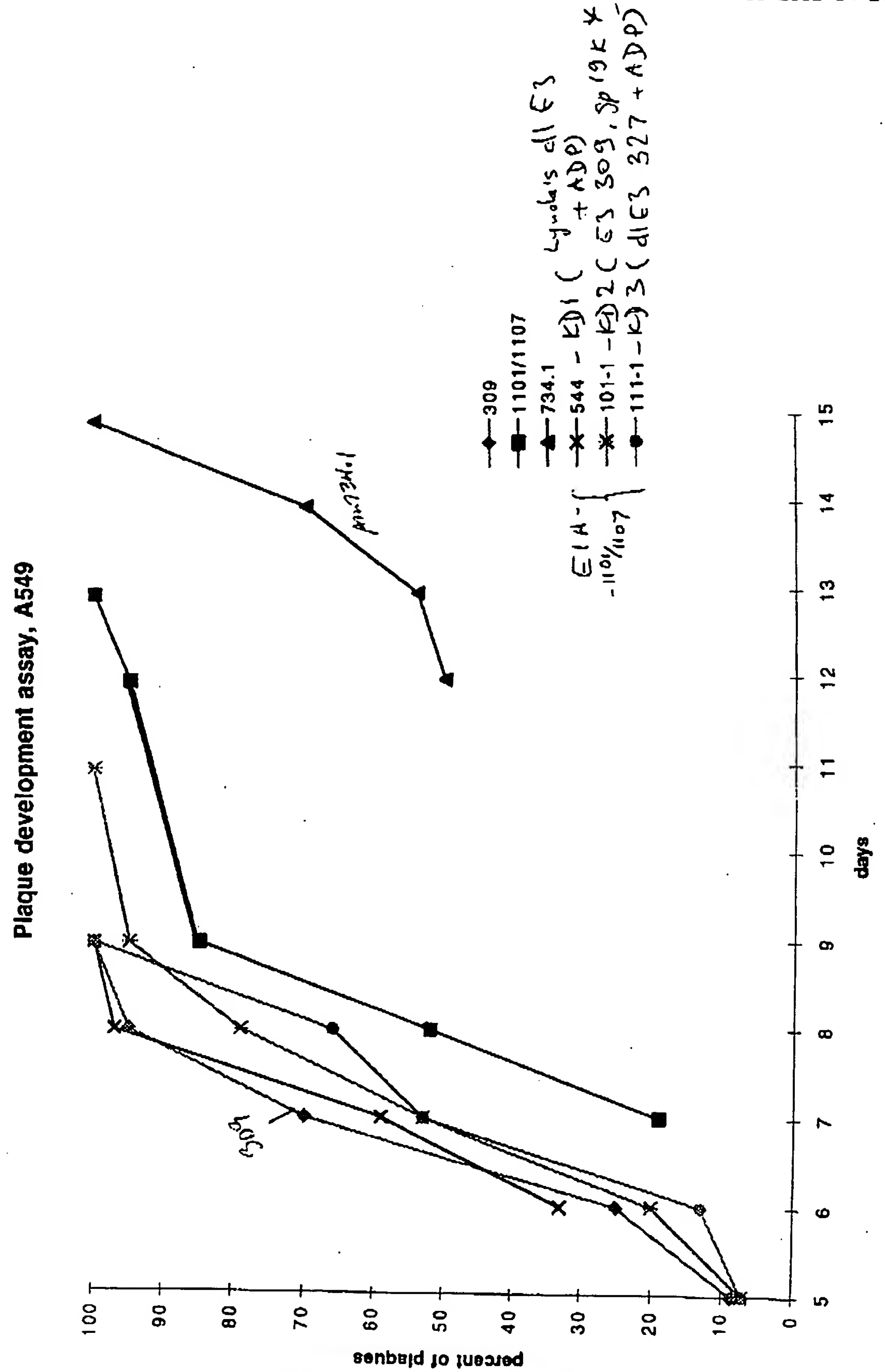
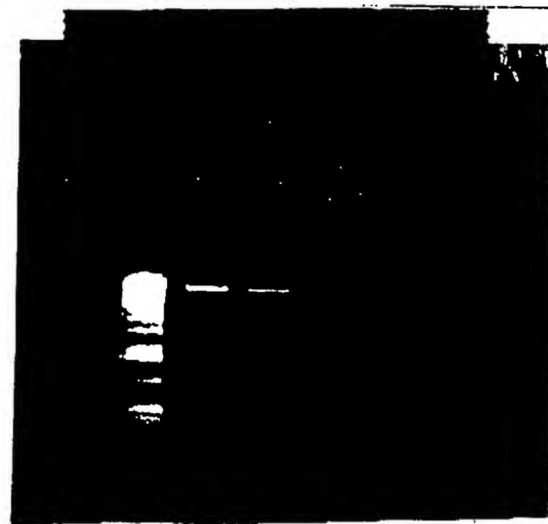

Plaque development assay, A549
percent of plaques
days
309
1101/1107
734.1
544
101-1
111-1

EXHIBIT
C66

Mohan, 6/13/97



1. C.F. project:

a) 293 cells expressing anti... by pΔE1Sp1A/3.7 SPC Rep78 a... Viral DNA – shows the f... slow.

b) 293 < Rep78 cell lines sho... fragment by PCR.

2. E3 project:

a) Particles/OD, OPU

	OPU[1]	OPU[2]	pfu
r700 =	1.52×10^{12}	9.8×10^{11}	9.88×10^{10}
C+14.7 =	1.9×10^{12}	9×10^{11}	–
GP19K =	–	2.8×10^{11}	1.2×10^{9}
ITR LacZ =	–	2.2×10^{12}	9.8×10^{10}

PFU will be ~ 10^7 reproducible

EXHIBIT C67

b) CsCl purified r700, C+14.7, and GP19K virus – 100 μl → Viral DNA → gel.
It seems from the gel, OPU/DNA ratio looks reasonable.
If it is true then virus prep = does it have more defective particles in C+14.7, or Toxic?

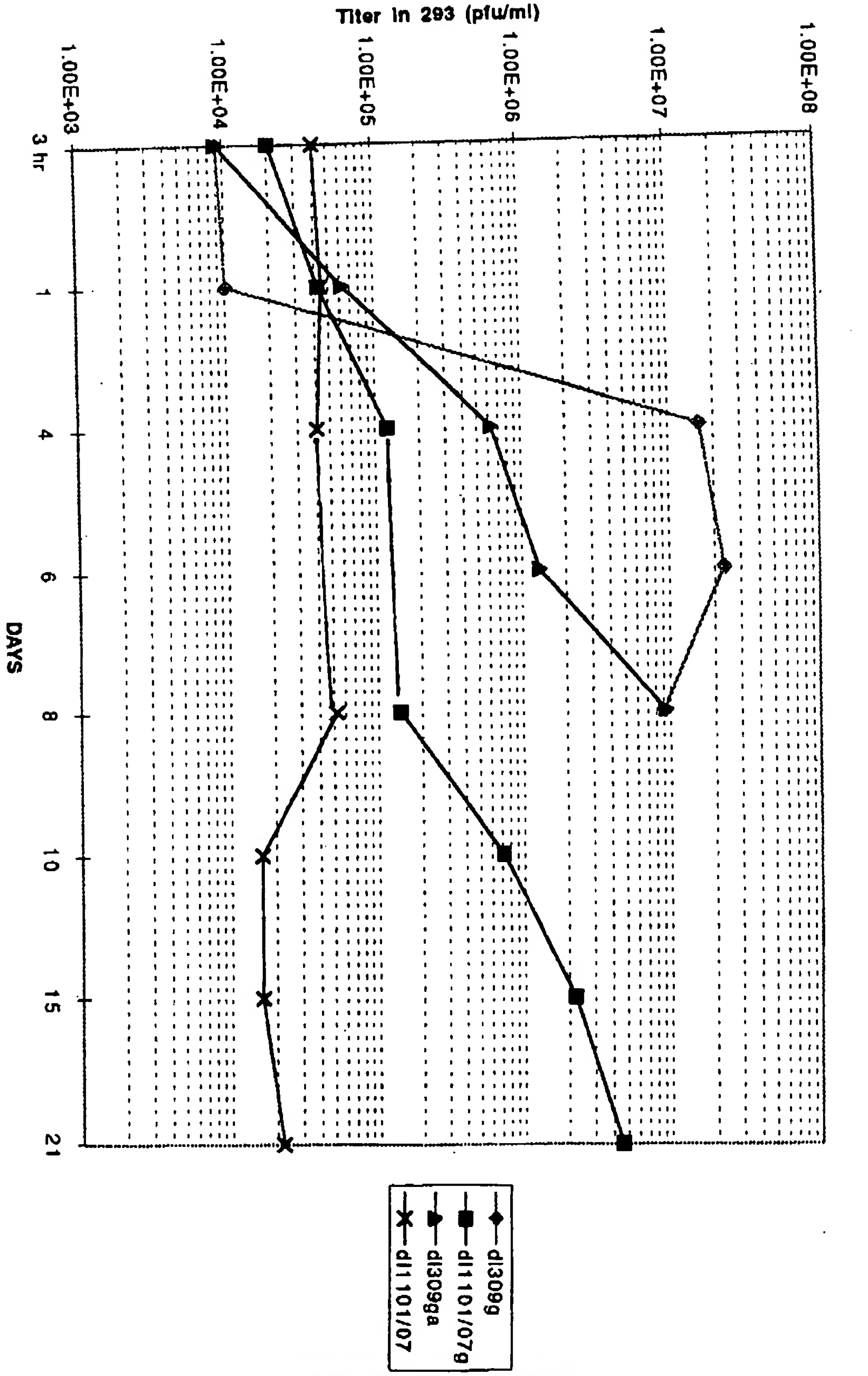
c) I.F: A549 cells, r700, NPGK 14.7 and GP19K (50 pfu/c...) & 14.7.

d) Transferring gp19K fragment from pCI 19K to left hand plasmid pΔE3p1A and p#30 (pΔE1Sp1/VA1 ...
#30 NPGK gp19 → pCI Have clone pΔE1Sp1
AP (LacZ opposite direct...
I have a clone in pΔE1Sp1A → plasmid preps →
I have clones in p#30 vectors → analyzing
VA1, VA2, lacZ

3. Cancer project: MEL 299 cells: dl 309 and dl 1101/1107 (500 pfu)
See growth curve graph

Sheet1 Chart 6

Growth curve 500 pfu/cell



EXHIBIT

C68

Karoly 6/10/?

E3 proteins

- I did a Western with Ct 14.7 and VPGK147B
- I am doing an IF with Kostya's CMV E3 and Todor's 760 RID
- I have a problem with cloning, I am working on it.

FasL project

- I have ≈ 15 new plaques, they came at about the same time as lacZ. I am growing them up.

Cancer therapy

- Time course in 293, A549 and MCF 7 is done, I am making 0 hour timepoints.

E2-Gal

- No plaques yet (not even in control).

EXHIBIT
C69

Kostya 6/11/97

1) E3 project.

734.1 ← E3

i) Have made the left-hand plasmid based on p#30 (pΔE1sp + lacZ-RSV) with CMV-E3 pm 734.1 expression cassette in it. Now making CsCl prep. to check for expression and making viruses.

ii) Failed to clone same CMV-E3 pm 734.1 into plasmid with Lynda's E3 deletion. Going to reclone CMV-E3pm 734.1 into pCI 2xPac (Lynda), then I'll be able to put the expression cassette to Pac I site of either Lynda's plasmid or pBHG11.

iii) Karol have shown that Terry's PCI/14.7 either don't express or express weak. I'm going to clone 14.7 from pMT2/14.7 to pCDNA3.1Zeo(+), than CMV-14.7 to p#30.

EXHIBIT C70

) Cancer therapy project.

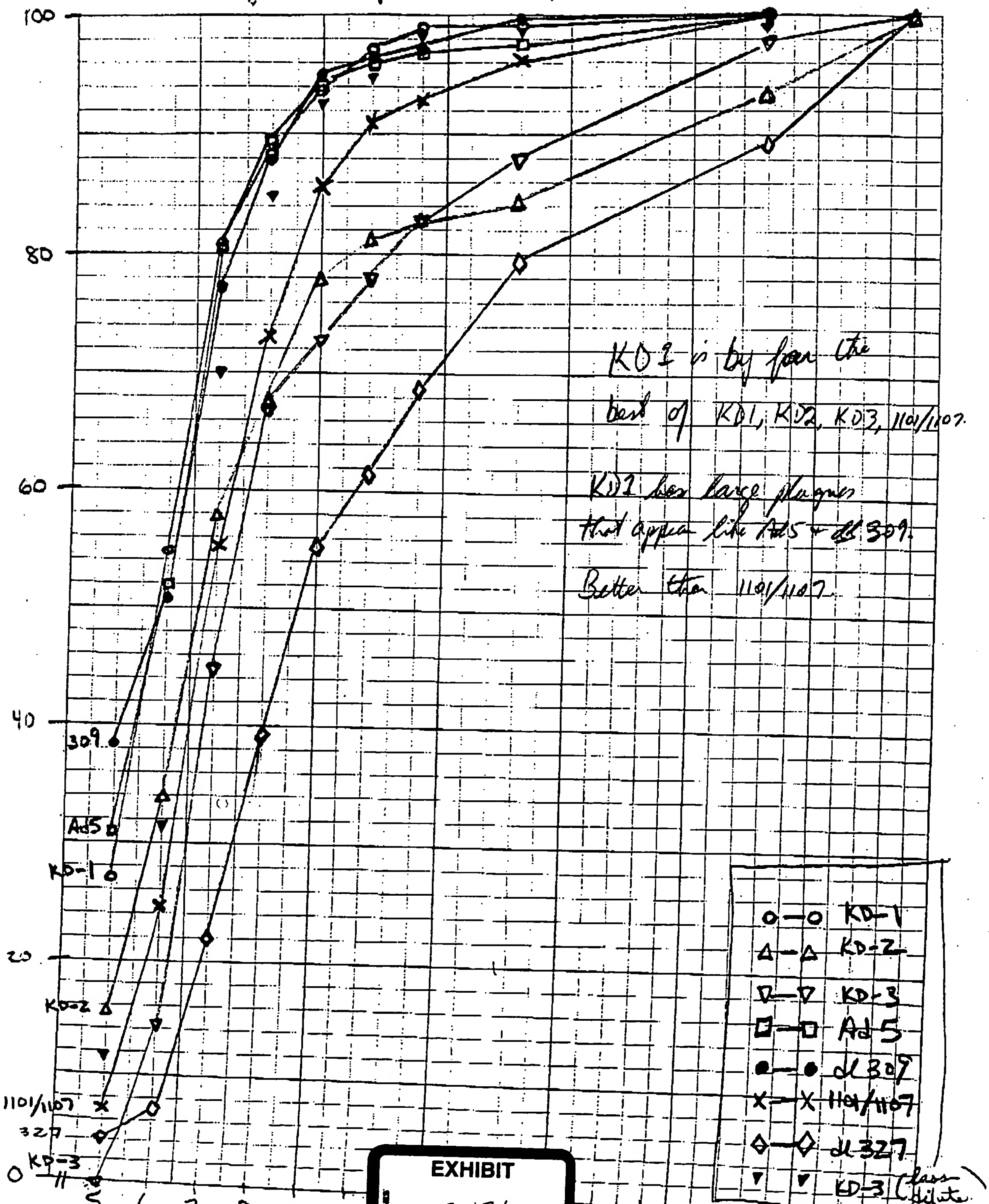
i) Got 293/E4 from Valery Krougliak. Now growing them up to freeze and to repeat transfection experiments with SP-B ↔ E4 promoter substitution.

→ Plaques are later than E4(+)
→ plaques are of different morphology (dl366 for me as well)
→ Defect of linearization?

ii) 293 cotransfection of p626 E4⇔CMV + dl 366.
293 cotransfection with E4 expressing plasmids.

iii) Ann's results with KD 3

970617 Plaque Assays on A549



EXHIBIT

C71

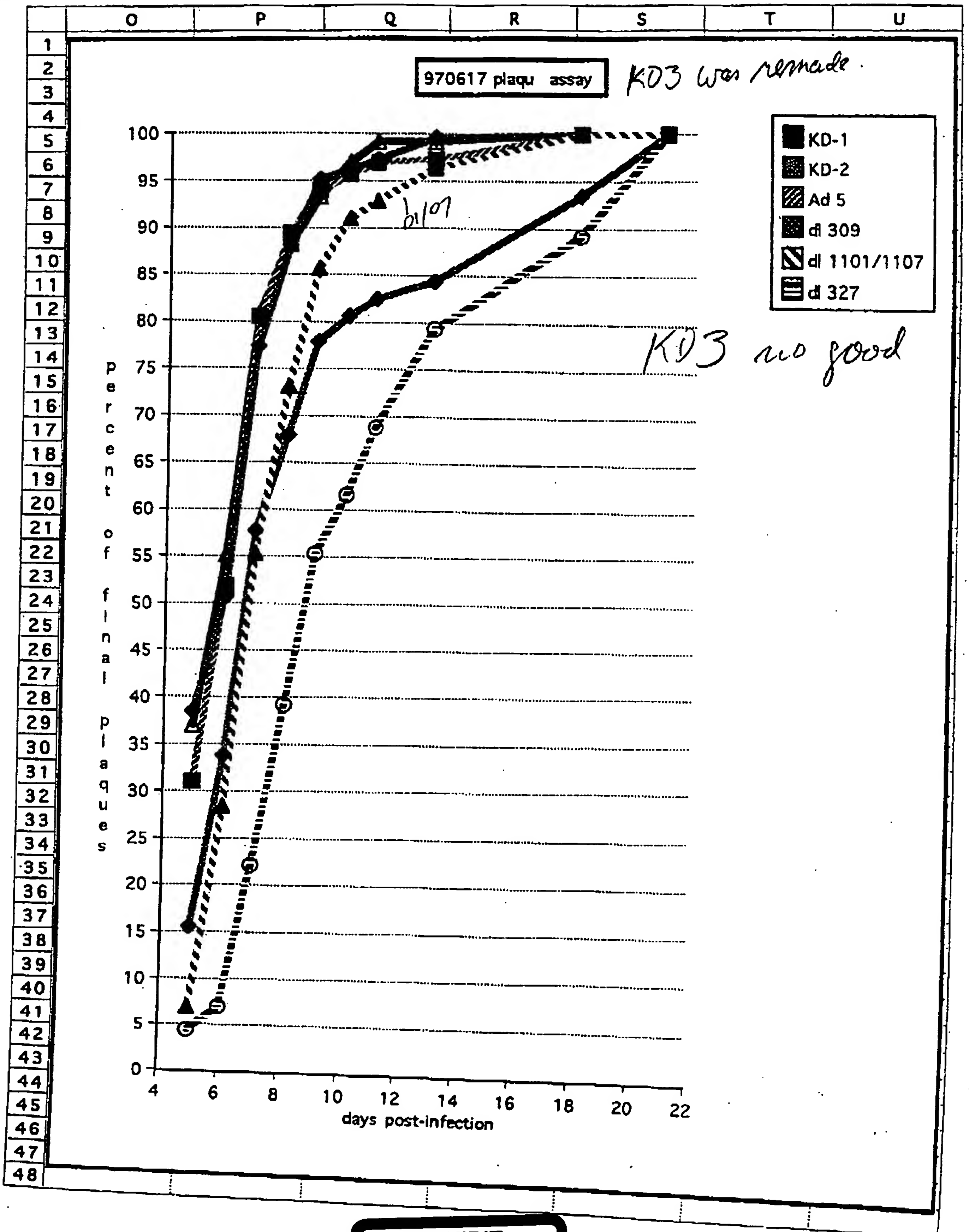

970617 plaqu assay
KD3 was remade
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
percent of final plaques
4
6
8
10
12
14
16
18
20
22
days post-infection
KD-1
KD-2
Ad 5
dl 309
dl 1101/1107
dl 327
KD3 no good

EXHIBIT
C12

	A	B	C	D	E	F	G
1	970617 PA						
2	KD-1	5	6	7	8	9	10
3		37	55.2	80.9	88.4	93.4	97.1
4	KD-2	5	6	7	8	9	10
5		15.6	33.9	57.8	67.9	78	80.7
6	Ad 5	5	6	7	8	9	10
7		31.2	51.9	80.6	89.5	94.1	95.8
8	dl 309	5	6	7	8	9	10
9		38.5	50.8	77.5	88.1	95.1	96.3
10	dl 1101/1107	5	6	7	8	9	10
11		7.1	28.6	55.4	73.2	85.7	91.1
12	dl 327	5	6	7	8	9	10
13		4.5	7.1	22.3	39.3	55.4	61.6
14							
15							
16							
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EXHIBIT

C13

	H	I	J	K	L	M	N
1							
2	11	13	18	21			
3	99.2	99.2	100	100			
4	11	13	18	21			
5	82.6	84.4	93.6	100			
6	11	13	18	21			
7	97	97.5	100	100			
8	11	13	18	21			
9	97.5	99.6	100	100			
10	11	13	18	21			
11	92.9	96.4	100	100			
12	11	13	18	21			
13	68.8	79.5	89.3	100			
14							
15							
16							
17							
18							
19							
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EXHIBIT
C74

06/20/97 Kostya

1) E3 project

i) Started cloning of CMV-E3 pm 734.1 expression cassette to left-hand plasmid (p#30) and into E3 (in place of Lynda's E3 deletion).

→ Clone into SPC expression cassette the TG module.

has zeozine pcDNA3.1zeo+

ii) Started cloning of CMV-14.7 expression cassette from pe1-14.7 (Terry-Chip) to left-hand plasmid.

2) Cancer therapy project

Have no plaques with SPB ⇔ E4 promoter substitution. (293-TTF1 Mohan)

I'm starting with Hep/E4, A549/E4 from GTI, need to check them for ability to support growth of 366 (E4dl).

W162 - (expressing E4) - if Ann can find them.

Growing up plasmids containing E4 under the control of CMV and native E4 promoters. (for cotransfection)

Valeri Krougliak wrote me that he would be able to send us 293/E4 (VK2-20) when Dr. Wold write him a letter with request. His address is on the next page.

TI-E4 deletion cassettes.

EXHIBIT

C75

Karoly 6/20/97

E3 proteins

9p19K, pol II in E3 9p19K, pol II trunc in E1

- I am growing up KT2 and KT9 into the 15 cm dish
- I am doing a Western with KT4 and KT6
- I am doing a time-course with KT7 and Ct (KT/1?)
- I am cloning R10-s with CMV promoter into the shuttle vectors Have in Nripen's shuttle, Graham Shuttle. R10 from Ad2 and Ad5.

- MT2 promoter is in pCI2Pac (Lynda) (maybe: probably)

~~Fool project~~

- I am growing the ~~clones~~ plaques Have 20 plaques. These plaques grow very slowly in 293/CrmA cells.

CrmA

I have CrmA in Nripen's shuttle plasmid, I am going to make virus with it.

Cancer therapy

- I am titering missing timepoints.

CMV-14.7 (Kathy)
CMV-9p19 (Mohan)

We need maps and sequence of plasmids ~~that~~ they sent us. Kostya will check. Especially the left hand plasmid with RSVLTR/LacZ

Given to GTI 6/18/97
pCI-R10(5)
pCI-R10(2) } Chip
pCI-9p19K

Will send to GTI next week.
pCI-14.7K (Lexgrosmu)
Send to GTI next week

EXHIBIT
C76

Valeri Krougliak,
Assistant Professor
Institute for Gene Therapy
Mount Sinai Medical Center, box 1496,
One Gustave L. Levy Place
New York, NY, 10029
Tel. 212-824-7748
Fax: 212-849-2437
E.mail krougv01@doc.mssm.edu

293 expressing E4 (VK2-20)

Published in Human Gene Therapy 6: 1575-1586 (December 1995)

Have 3 plasmids with E4 deletion.
Cannot rescue virus.

EXHIBIT
C77

4/27/97 Kosiya

1) E3 project

i) Cloning of CMV-E3 pm 734.1 expression cassette into left-hand plasmid and into plasmid with E3 deletion is in progress.

ii) Terry's pCI-14.7. Made miniprep of the plasmid, it looks like this is pCI with Ad5 14.7 in it. Made maxiprep, Karl is checking it for expression. Now doing recloning of CMV-14.7 expression cassette from this plasmid to left-hand plasmid.

2) Cancer therapy project

Got A549/E4; Hep/E4; W162 from Ann. Doing plague assay of dl366 on these cells with 293 and A549 as controls.

Going to try to optimize transfection efficiency for these cells.

Going to try transfection-infection experiment (infection with 366, transfection with CMV-E4, SPB-E4 & E4-E promoter as a control; on 293 cells.

Going to try cotransfect 293 with 1101/1107/EcoRI + + CMV-E4 plasmid).

293/E4 (Valeri Krougliak)?

EXHIBIT C18

PINE 3.95 MESSAGE TEXT Folder: MAIL Message 41 of 42 90%

Date: Mon, 23 Jun 1997 17:28:35 -0600 (CST)
From: WOLDWS@SLU.EDU
To: "KROUGV01@DOC.MSSM.EDU"@SLU.EDU
Cc: WOLDWS@SLU.EDU
Subj ct: Request for cells

Dear Valeri,

I hope all is going well with you. I'm happy to see that you have a faculty position in a good university.

I am writing to request the 293 cell line expressing E4 (VK2-20) that you published in Human Gene Therapy (6:1575-1586, 1995). We have several plasmids with deletions in E4, and we have been unsuccessful in rescuing them into virus. We would use your cell line for that purpose.

Thank you in advance.

Sincerely,

? Help	M Main Menu	P PrevMsg	- PrevPage	D Delete	R Reply
O OTHER CMDS	V ViewAttch	N NextMsg	Spc NextPage	U Undelete	F Forward

EXHIBIT
C79

970687 Gene Therapy Meeting

(1) Fas L Ad Karl
– 13 of Fas L vector ~ 11 blue (23 plaques total)
1 blue + killed MCF7-CrmA

3 plaques by PCR do have Fas L gene.
pCI-FasL in Nrippons left end shuttle. pBHG 11
Next 5 from 60 mm dishes
Check by IF – all plaques.
Check by PCR for Fas L.

(2) Ad-14.7 PGK vector – expression at 4 d pi
p734.1 – gp19 exp., 14.7k exp.

(3) C+ kills cells at 2 days pi. CsCl stock – strange discrepancy between particles + titers.
Raffik → supernatant → perhaps some expression.
this plasmid expresses β gluc in transient/COS cells.
β-actin promoter c out enhancer; pol II for 14.7k.
this was done at 10^8 PFU (titer could be much higher)
Repeat using titer calculated from OD + DNA agarose gel.
This was better than β-actin promoter enhancer in COS.
Karl tried to make vector from several diff promoters/βgal;
this was only one that gave plaques.

D... S... ... ...

.GEL 1997:06:27 12:46:35, Range - 0.01-249.86 Counts, 1.00x

Karl

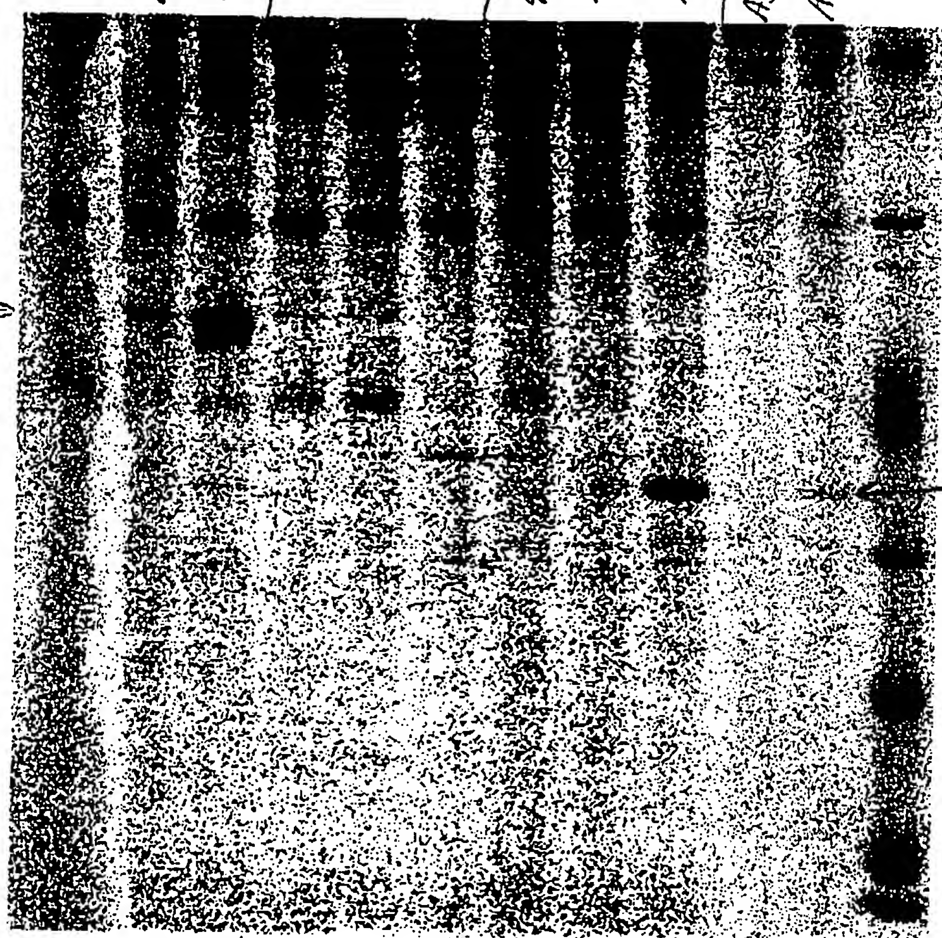
Transfection of p734.1 into 293

exposure overnight on PhosphorImager.

14.7 is prob mod.

293 | 293 + 734.1 | 700 (90 h pi) | 293 | 734.1 | 700 (30 h pi) | 293 | 734.1 | 700 (30 h pi) | A549 | A549 + NPCK-14.7B day 3 pi

gp19K →



— 14.7K is mod

α gp19K	+	+	+								
α RID β				+	+	+					
α 14.7							+	+	+	+	+

EXHIBIT

C81

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Dec. 19 2002 12:47PM P13

Mohan, 6/27/94

1 C.F. project:

a) 4 plaques (SPC Rep78 virus?) are growing in 293 ⊂78 cells. 3 plaques showed (very good CPE) CPE. 4th one is slow ~ will be fine.

b) RT-PCR for Rep78 antisense RNA were done employing RNA ± Reverse transcriptase to verify the PCR fragment is due to RNA and not from DNA contamination. This work — See graph.
It seems 293 ⊂Rep78 cell line is expressing Rep78 antisense RNA.

c) pSub VIII plasmid → chips → plasmid preparation. Elizabeth will make.

2 E3 project:

Particles / OD = O[...]

a) I got six viruses from [...]

	pfu/ml	OD	OPU
+ dl704	2.4×10^{11}		~~[illegible]~~ 0.[...]
dl722	1.35×10^{11}		0.99×10^{12}
dl731	1.44×10^{11}		1×10^{12}
dl739	7.7×10^{10}		$1.72 \times 10^{1[...]}$
dl753	1.07×10^{11}		$1.18 \times 10^{1[...]}$
dl762	1.58×10^{11}		$3.18 \times 10^{1[...]}$

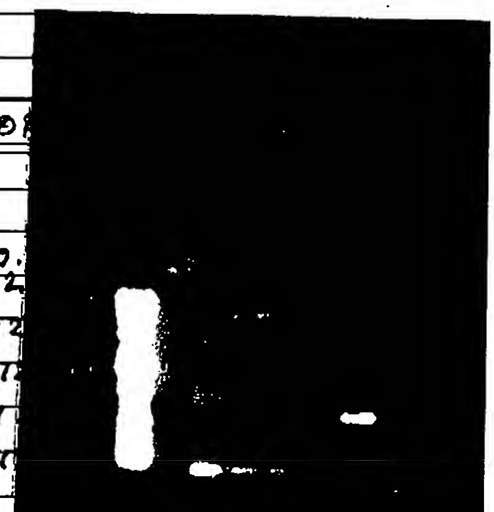


EXHIBIT C82

dl704 OPU is lesser than pfu. (Repeat
- do 10^8 pfu + 10^9 pfu

b) MCF-7 cells transfected by pEISpIA/gp19K and p#30 (Nimpen's) gp19K (#2, #3 & #7) got contaminated. (Yeast) Repeating again for I.F.

c) Cloning Ad2, Ad5 RID into pEISpCA and p#30 is in progress (from Karl)

d) KTI (14.7 LacZ) gave 6.2×10^6 pfu → More CPE
Stocks → Sinking [...]

EXHIBIT

D

1402 South Grand Blvd.
St. Louis, MO 63104
Phone: 314-577-8432
FAX: 314-773-3403

SAINT LOUIS UNIVERSITY

Department of Molecular Microbiology & Immunology

Health Sciences Center
School of Medicine

Facsimile Transmittal

Date: 12-19-02

From: William Wold

Phone: 314-577-8432 Fax: 314-773-3403

Deliver to: Dan Kasten

Phone: ________ Fax: 552-7305

Total number of pages including this page: 15

Message:

8 - 100 mm

7/13

todo

Phone:

Ab in whole / read [illegible] on Arms desk

Inventory virus book * [illegible]

3 days [illegible]
Use [illegible]
7001

(K.Bs from freezer) / when brought up

2×10^5/ml

Develop the gel (Monday)

7.5×10^7

(Cells frozen + thrown out) ✓

[illegible]

3mls / 250ml cell

Ad 1 + Ad 6 virus stock (5mls of stock)

Set up
1-100ml → Stock

11.6 pre absorbed. ✓ Set up

293 [illegible] Kay [illegible] CK

Ginsberg A5 [illegible] III (Layered stock) (175 flasks)

VS 103 [illegible] stock 50ml → [illegible] plate → [illegible]

5-4 [illegible] Spin [illegible] / medium 20% FBS 100ml → [illegible]

(Harvested) (960725)

Bayley 11/07/6 (911003) 104.8 [illegible] 50 [illegible] → [illegible] plate

~~freeze thaw~~

harvested (960725) 104-8

EXHIBIT
D1

9/4/96

CsCl Banded the virus Ad1 960825
1101/1107 Baileys (960802)

1 freeze/thaw
2 sonicate X 2
3 Vol after son 1101 < 21mls

Ad1 < 19mls

add $V \times 0.51 = g$ of CsCl

10.71g in 1101/1107 Baileys
9.69g in Ad1.

infect 3 liter Spinner with
dl 717 Vp 891208 Titer 1.3×10^{11}

cell count 3liter 3.5×10^5
Vol = 3liters (3000mls)

$3.5 \times 10^5 \times 3000$mls 10.5×10^8 total cells

use 20 pfu's / cell

$.162ul = \dfrac{.0105 \times 10^{11}}{1.3 \times 10^{11}} \times 20$ pfu's

use

KB's are healthy no clumping.

EXHIBIT
D2

9/17

1. Atlanta 6013E first (cells are shriveled up)
$\frac{19}{4} \times 3 =$ 1.4 x 105

* 2. Browhitakee cells look very health
6m1228
$\frac{28}{4} \times 3 =$ 2.1 x 105

3. nyc Apa 4819 nice + healthy cells very fat + round
$\frac{31}{4} \times 3 =$ 2.3 x 105

4. present 4m1938 cells are dividing now
$\frac{21}{4} \times 3$ smaller cells but healthy
1.5 x 105

5. nyc AF B4870 cells look great
$\frac{22}{4} \times 3$ 1.6 x 105

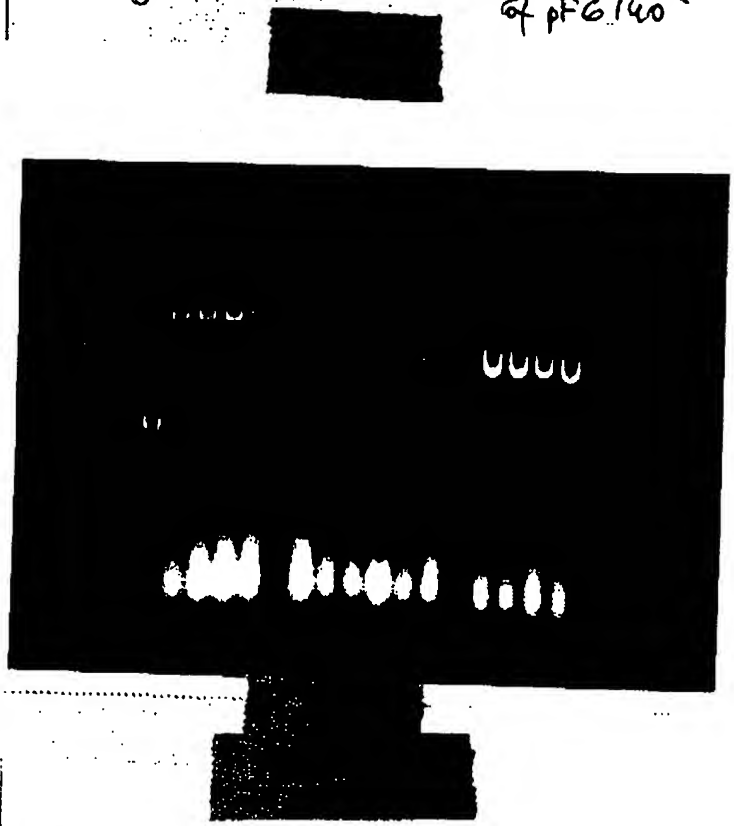
6 B10W 6m0741 strange shaped cells
$21/4 \times 3$ Rough edges not round
1.5 x 105

Infected 5 liter spinner with 1101/1107 20 mls
3.7 x 105 cells/ml

EXHIBIT
D3

September 18, 1996.

Cloning EcoRI - Xba B fragment of pF6140 to pBSSK(+)/EcoRI + XbaI



Lanes: minipreps of:

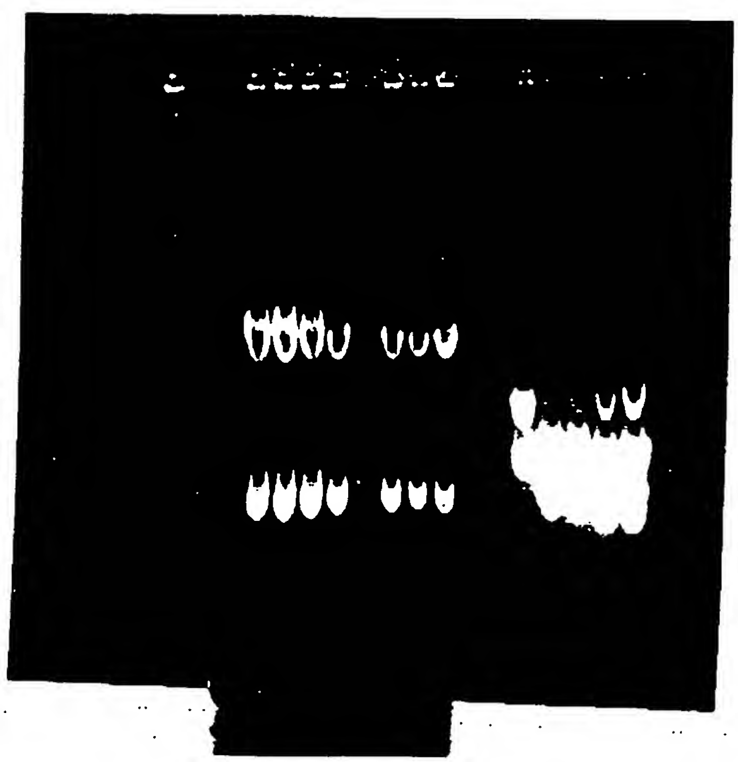
1) pBSSK(+)

2) - 9) clones

10) - 12) p21 (Nrippen's plasmid

14) - (7) pISP-C3.7.

First experiment 09/18/96



Restriction pattern of the plasmids.

1) Ladder

2) vector (pBS KS(+) RI/XbaI)

3) fragment B pF6140/RI/Xb

4) - 11) - RI + XbaI clones 1-8.

13), 14) - p21 (Nrippen) EcoRI

15) - 18) - pISPC Nde + EcoRI.

pBSKS(+) + 8 fr. of pFG/UO K1/ Tlg

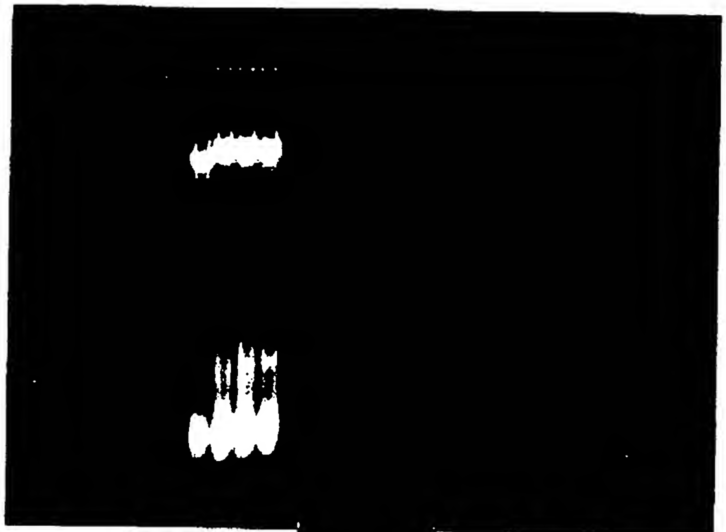
(pK1)

lanes: 1) ladder
2) RI + NdeI
3) Bst 1107 I
4) Sun I
5) Bst 1107 I + Sun I

multi core

⇒ size of RI - Nde fragment?

⇒ DNA masked 600 bp ~~K~~
Bst 1107 I - Sun I fragment



2% agarose gel
1) Ladder
2) pK1 / Sun I + Bst 1107 I + RNAse
⇒ ~600 bp fragment

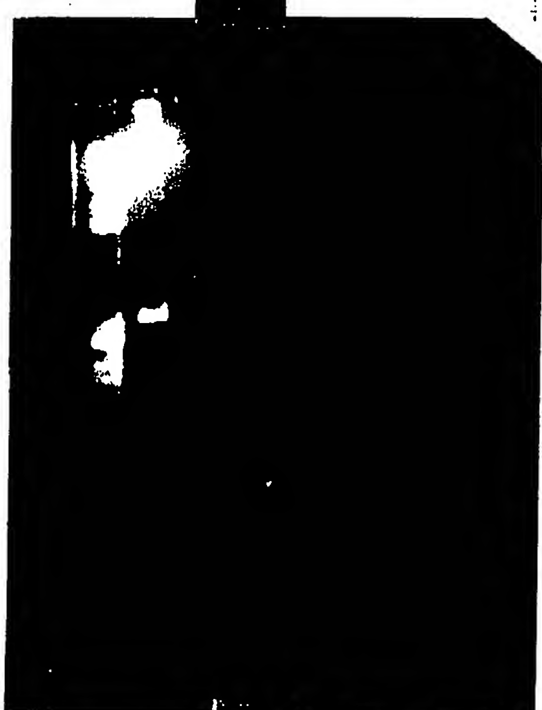
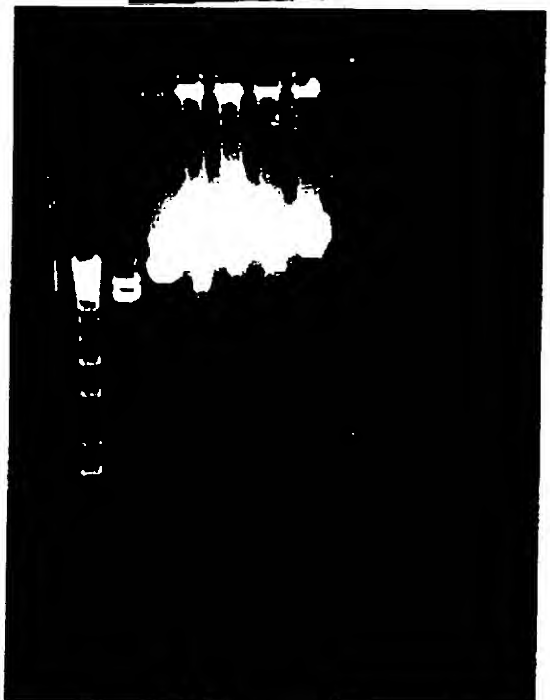


EXHIBIT
D5



Checking the restriction pattern of plasmids

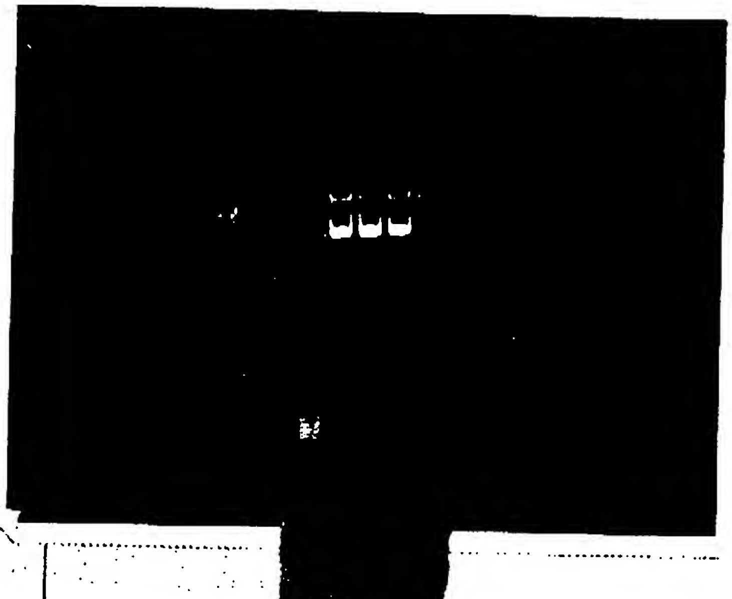
m. Ladder
2) L 1 / Nde + RI
3) L 1 / Xba
4) L 2 / Nde + RI
5) L 2 / Pac I
6) L 3 / Nde + RI
7) L 3 / Pac I

EXHIBIT
D6

28 September 1996.

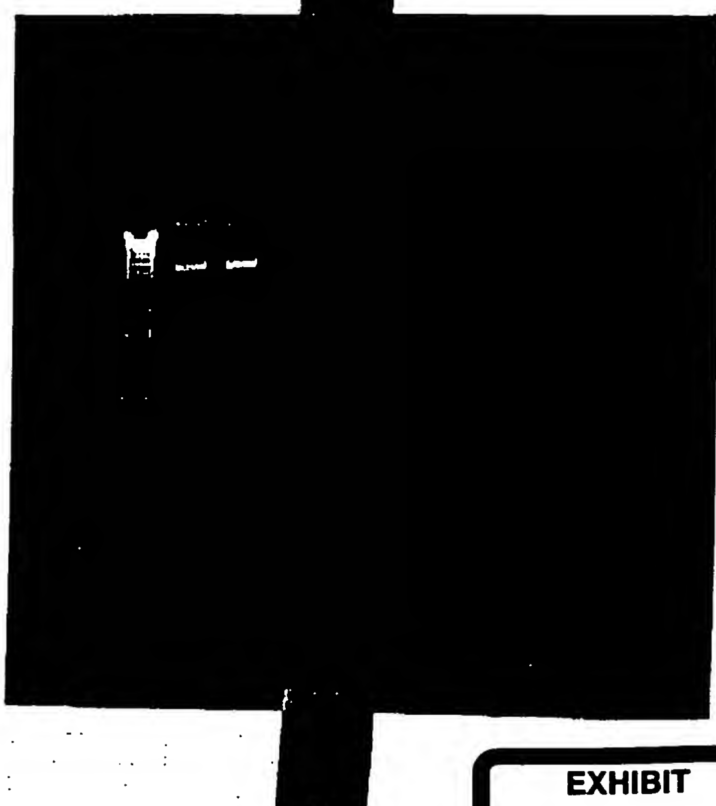
minipreps.

[redacted]



1) Ladder ul.
2,3,4) - pGSP-B 2.2
5,6,7) - L2 ⇒
⇒ pLKHE2A + Bam → end + Cla
8,9,10) - L3 ⇒
⇒ pCR(LKH dl)Bam → end E2A+

SPB experiment
10/27/96



minipreps.

1) Ladder
2) - 3) - L1 ⇒
⇒ pT~~Z~~Ad - Xba.

September 30, 86.

Cloning of CMV promoter into Bst 1107I site of p21 (Nrippk).

1) ladder
2) p21 / Bst 1107I
3) pCDNA3 / Bgl II + BamH I

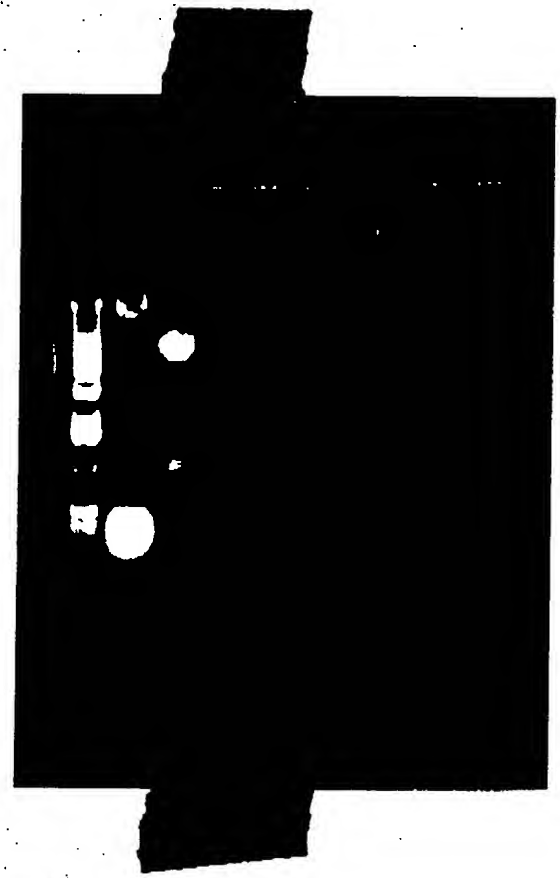
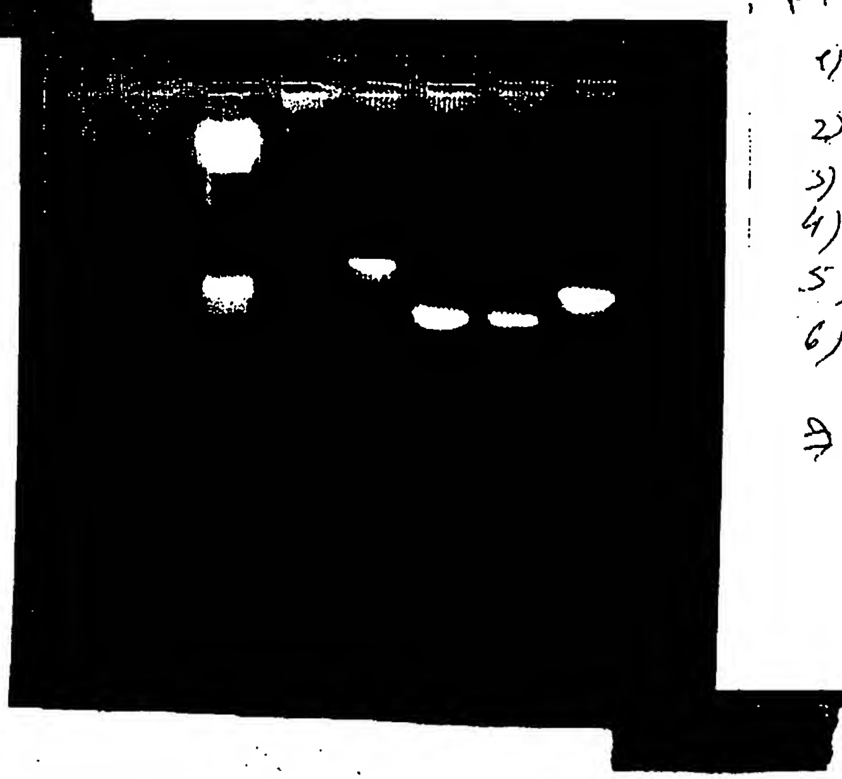


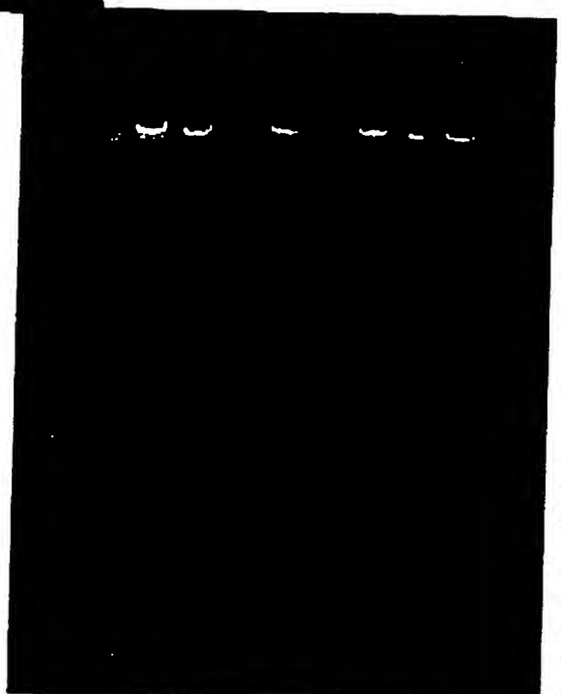
EXHIBIT
D8

October 2 86 Testing primers in PCR.



1) ladder
2) KD2 - KD3
3) KD1 - KD2
4) KD4 - KD5
5) KD6 - KD7
6) KD8 - KD9.

→ all 9 primers are OK.



October 9, 96

Analysis of clones 1-2, 4-8 of pK1/BstNotI + SauI + CIP + 19C* (with mutation)

1) ladder

2) pK1 / RI + PacI

3) - 9) - clones / RI + PacI

⇒ clones 1, 4 - 8 ⇒ recombinant

⇒ clone 2 - wt.

⇒ name recombinant plasmid pK2

1

October 11, 96.

Analysis of minipreps by restriction

~~ladder~~

1) pK2 / SacII

2) - 8) clones 1 - 7 / SacII

9) - ladder.

pK2/PacI ADP ORF (PCR) with PAC sites at ends

⇒ 1, 4, 7 - recombinant orientation ?

Cloning KD1 ADP
10/11/96

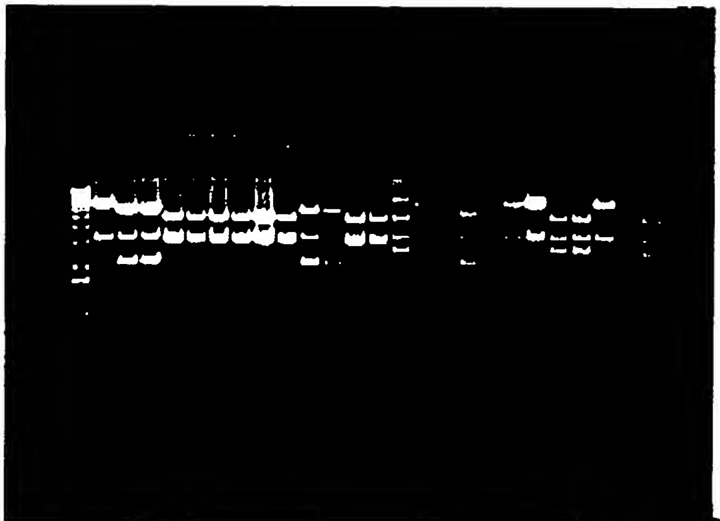
EXHIBIT D9

October 27, 96.

[redacted]

Analysis of mini preps by restriction.

1) ladder
2) p21 / KpnI
3.) - (14) clones
p21 / Bst + CMV prom.
Bgl + Bam + Klenow →
⇒ clones 125 - 136 / Kpn



[redacted]

clones 25, 26, 33, 34 ⇒ ~~the orientation that I need~~ (allowing transcription)

clones 27, 28, 29, 30, 31, 32
35, 36 ⇒ other orient.

+ clone 23 from other cloning is ok.

15 - 26) clones p21 / Bst + PCR product - - promoter SP-B (500 bp) with Bst I sites on the ends

⇒ clones 225 - 236 / KpnI

⇒ clones 27, 28 ⇒ correct orientation
+ clone 223 from preceding cloning.

clones 25, 31, 32 ⇒ other orientation.

Cloned SPB prom.
in E4 10/26/96

C primer ≈ 70 pmoles/µl

3 p1 1380 Sma I
1741 Kpn I ⇒ to sequence 3ex (1, 9, 10 *) use (+) 1 and (−) 3
200 1981 Bst I

Wednesday, October 30, 96.

1) 3ex Clone 1, 4(?) – new preparation for sequencing

√ 2) Purify ADP Xba fragment, purify pL1/Xba/CIP vector

Ligation insert (ADP Xba) vector L1/Xba/CIP
0.1 µg/µl 0.1 µg/5 µl

control 15 µl H_2O 5 µl vector

4 experiment 15 µl insert 5 µl vector

+ 5 µl buffer (5X) + 1 µl ligase → 1 hr room temp.

Thursday, October 31, 96.

1) 293 cells tomorrow will be ready to pass
⇒ prepare HEBS and $CaCl_2$ What about 2X media?

2) Sequence 1, 11

3) Prepare new ADP Xba insert! Ligate, transform!

4) Order primers for sequencing p21 inserts

3) first, repeat PCR with Pfu pol.

5) Ask Nathan about TP-Drug AdS.

C = 7.8 ng/µl = 0.078 µg/µl

Cloning KD3 ADP

EXHIBIT
D11

Sequencing :

pK2 (gp1914*) ⇒ clone 1

- Mix — 8 μl
- Template — 1 μl
- Primer — 5 μl
(KD1 or KD3)
- H_2O — 6 μl

R.T. ramp 96°C

96°C — 30"
50°C — 15"
60°C — 4 min!

25 cycles ⇒ KD1 & KD3

HEBS 2x for 200 ml

- 2 g HEPES
- 3.2 g NaCl
- 0.148 g KCl
- 0.039 g Na_2HPO_4 (anhydrous)
- 0.4 g glucose

final pH = 7.0

$CaCl_2$ 2.5M
MW = 147.00
for 100 ml
36.75 g

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS

EXHIBIT
D12

Friday 1, November, 1996

1) Prepare HEBS, $CaCl_2$, test precipitate, filter
2) PCR of pFu, cut with XbaI, run e-gel, purify fragment, ligate, transform DH5α
3) Cut TP-DNA with EcoRI, check on gel.
no fragment ?? repeat, take pFG140 for template!!!
~~take~~ other pfu polymerase !!

Saturday 2, November, 1996.

1) Set up cells on 6 cm dishes 6 dishes.
2) Prepare HCl (0.1 N), NaOH (0.1 N) for cell culture.

O/N 3) Cut TP-DNA with EcoRI, run electrophoresis.
O/N 4) PCR AdXba with pfu, pFG140 as template.
5) filter sterilize Carol's carrier DNA (salmon sperm DNA).

PCR with pfu.
10 µl template (1 → 1000 pFG140)
2 µl primer (4)
2 µl primer (5)
180 µl H_2O
20 µl buf for pfu.
2 µl pfu
4 µl of NTP.
200 µl.

Sunday 3, November 1996.
no PCR product, buffer pFu?
1) Cut Taq PCR product AdP-Xba, with XbaI.
2) Transfection
pFG 140. — 20 µl (10 γ)
each TPDNA 5 µl + carrier — 10 µl (10 γ)
on 2 dishes TPDNA 5 µl + pL2 20 µl (20 γ pL2XbaI)

EXHIBIT D13

Monday, 4, November 1996. (la very small volume!)

1) Purify fragment ADP-Dbs, ligate, transform DH5α.
2) When transforming DH5α, seed cells on SOB-agar for preparing competent cells.
3) Order primers for sequencing p21 insert.

Ligation { vect (L1) = 0.1/1
insert (ADPCR) = 0.01/5.

Ligation (promega)

control 1.5 μl vect + 8 μl H_2O + 1 μl ligase
exp. 1.5 μl vect + 8 μl fragment + 1 μl ligase } → 2h room temp.

! Sequencing failed ⇒ too low peaks!!!
Repeat with both primers 3x more template
3x more primers

mix → 8 μl
template → 4 μl
primers 1,3 = dilute 1/10
take ① - 2 μl (3)
③ - 4 μl (5)

+ 6 μl H_2O (1)
+ 4 μl H_2O (3)

!! There are no E3 poly A sites in L2!
May be it can use fiber's gene pA?
Moreover, there are no acceptor splicing site in your construct, how can it be expressed?

Check literature on expression of inserts in Xla deletion of E3.

Splicing in Ad, esp. E3 splicing.

Tuesday 5, November 1996.

1) Repeat sequencing: 3ex 1 clone primers 1 and 3
2) Seed clones new 4ex (5 clones) on sectors.
3) Prepare competent cells DH5α
○ 4) Check CMV promoter insert into p21 with other restrictases, start clone it into pBHG11.

Sequencing → with primer 3 have a precipitate, with primer 1 precipitate is much less

Wednesday 6, November 1996

1) Extract plasmids from clones 4ex (5 clones), check if they are recomb.
○ 2) 4) - from yesterday.

Thursday 7, November 1996.

1) Seed clones too liquide culture for minipreps with Wisard plus minipreps kit for sequencing
2) PCR with pfu of ADDXba (primers 4, 5)

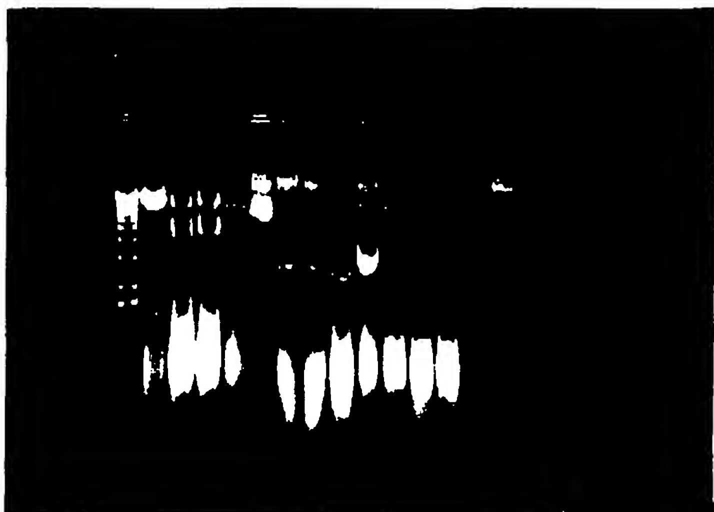
- 75 µl - H_2O
- 1 µl - template pE6 luc.
- 10 µl - buf pfu.
- 1 µl - primer 1
- 1 µl - primer 2
- 2 µl - dNTP.
- 1 µl - pfu
- 10 µl DMSO

3) Prepare vectors L1/Xba and pBHG11 / Sal I → large fragment
4) Check CMV in p21 (Nde I)
5) Check new vector DNA for MW?
6) PCR of inserts into p21 - with SP-B it is possible to check orientation!

EXHIBIT
D15

Nov 11, agarose gel electrophoresis.

1) - ladder
2) - 21
3) - 123 } cut (Buffer D, orange).
4) - ms
5) - L1
6) - 10) 1-5 clones resp.
11) - 14) L1/Xba prep
15) - pBu611 / R1 prep.



Conclusions!
1) it's ok with CMV promoter / use
2) it's ok with pBu611 / R1
3) Lex clones → trash
4) L1/Xba → trash.

November 10, 1996, Sunday., November 11, 1996, Monday

1) Split 293 cells on new flask.
2) Overlay 293 dishes with neutral red.
3) Run electrophoresis of Wizard minipreps and L1/Xba.
4) PCR of clones + electrophoresis
5) ~~Cut~~ CIP pBU611/RI cut from the gel?
6) Cut p21/CMV with EcoRI, cut the fragment from the gel?
7) Change the medium in 293 flask.
8) ~~New prep. of L1?~~ Clean up old preps?
9) Send 54, 57 and L1 for Wizard miniprep!

Tuesday 12, 1996

Cannot cut [illegible] after Wizard kit minipreps?
1) 123 - with EcoRI - [illegible], take from gel large fragment!
2) L1 - with XbaI ?

Wednesday 13, 1996.

1) CIP pBU611, cut fragment from gel, cut fragment 123/R1
2) Try to cut L1 with Xba, if not successful → seed L1 O/N
3) Ligate from 1)

12 µl vect + 5 µl insert + 1 µl enzym
+ 5 µl H_2O - control

L1/Xba not in single band ⇒ 1) O/N digest with XbaI
2) extract with Wizard ⇒
→ seed it in [illegible] culture

pBU611/R1/CIP - A - fragment - not single band ⇒
be ready to distinguish between all this forms.

Thursday ~~Tuesday~~ 14, 1996.

1) Transform DH5α with ligation mix from yesterday
(Try to obtain high efficiency!!!)
2) Extract L1 with Wizard, cut Xba, run together with yesterday's L1/Xba (one)! on 1% gel.

→ Try PCR! Maybe you can use it for screening ?!?!
→ Sequencing with new batches of 31!!!

PCR (Taq polimerase) for 150 µl
- 15 µl buffer
- 3 µl dNTP
- 4.5 µl $MgCl_2$
- 1 Taq µl
- 130 µl H_2O

1) 123 } primers 10, 11
2) 125
3) 223 } (insert in p21) — 6(8) 225 } primers 8, 10 — 7(9) 227 } primers 9, 11 → check orientation
4) 227
5) p21 - control (-)
10) 54 } primers 6, 7
11) 57
12) pL2 - control (-)

(13, 14, 15) — cut 54, 57 with PvuI; L? - control.

Σ 12 reactions — 13 (9-11); 14 (8-10); 15 (9-10)?; (6-7) — 225, 227 with BclI [illegible]; p21 - control.

EXHIBIT D17

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Health Sciences Center
School of Medicine

Facsimile Transmittal

Date: 12-19-02

From: William Wold

Phone: 314-577-8432 **Fax:** 314-773-3403

Deliver to: Dan Kasten

Phone: ______ **Fax:** 552-7305

Total number of pages including this page: 15

Message:

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Dec. 19 2002 11:23AM P2

See explanation of the picture on previous page.

[redacted]

[redacted]

Conclusions: everything is fine
except that [illegible] didn't cut DNA
(because of buffer 4, I think).

Friday 15, 1996.

1) ~~Transform E1~~! Clean up L1 preps., try to cut with Xba
2) Seed clones of pRH611 + 123 (Cm?).

AMPAD 22-141 50 SHEETS 22-142 100 SHEETS 22-144 200 SHEETS

EXHIBIT D18

E!
MAC 299. W138

Friday (1:00/

November ~~Monday~~, 18, 1996.

1) Analyse clones (1-24) pBUG11/RI + CMV (123 clone)
Run non cut and then cut with NdeI!

2) Phone about SrfI.

3) Transform L1.

4) ~~Sequence~~ PCR

5) Prepare TP-DNA, TP-DNA/RI, Ad5 DNA, Ad5 DNA/RI

6) Split 293 to dishes for transfection

Sequencing.

✓ 8 μl pre mix
✓ 5 μl p31 (0.58)
✓ 1 μl primer (VJ 1)
✓ 6 μl H_2O

✓ 20 μl oil

circling.

pBUG11 gave 2 small fragments instead of 1.
~~check~~ what is it?
clones gave incomplete digestion → O/N NdeI
after PEG precipitation

Tuesday, November 19, 1996.

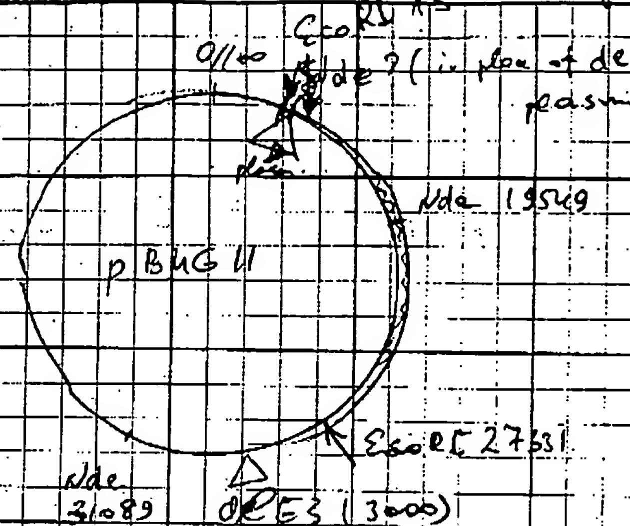
1), 3), 5), 6) from yesterday.
if fail in 1), do PCR with 10, 11 primers!

Freezer at CF room 3 shelf from top
left drawer
upper box (-4)
tube with 918 on cap
(VS 20) on side

→ Ad 5
CsCl.

EXHIBIT
D19

EcoRI is here if plasmid based on pUR



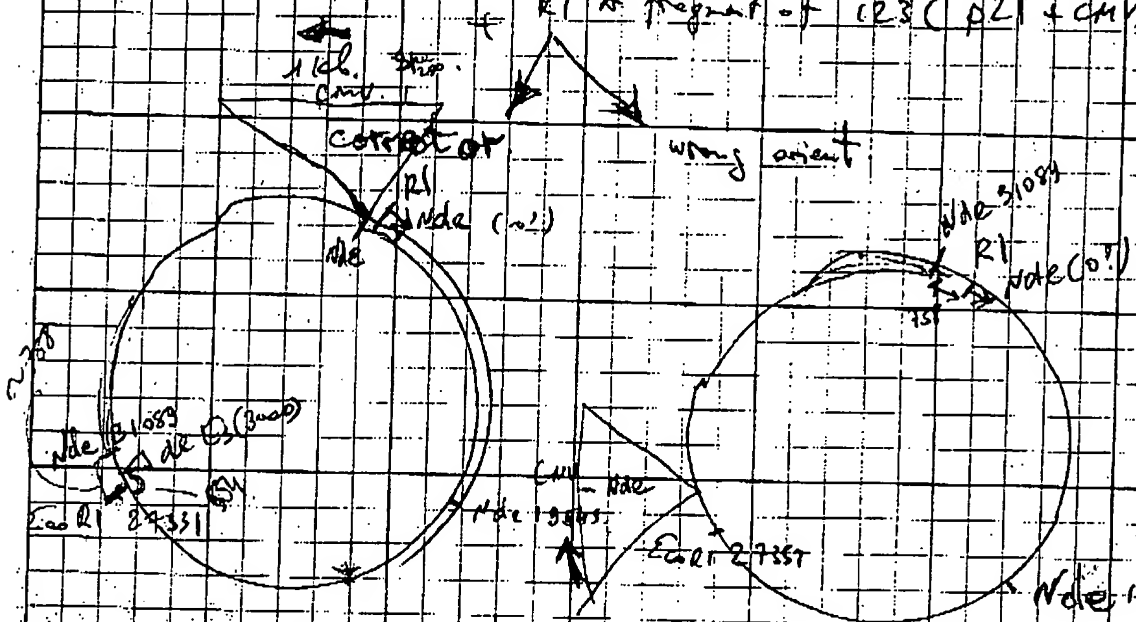
Nde I fragments

~ 18~~664~~

~ 85~~40~~

~ ~~6300~~ 7540

RI A fragment of rc3 (p21 + CMV)



~ 18~~900~~ same as wt

~ ~~8595~~ same as wt

~ 5000

~ ~~3000~~ instead of ~~4665~~

3565

8282

same

~ 18~~900~~ same as wt

~ ~~5000~~ same mw as wt or slightly differ

~ 5000

~ 3500 instead of 5000

1 - ladder

2 - cut 4 Os

26 - samples

50 13

~ 18254 same as wt

~ 8540 same as wt

~ 5000

~ ~~3590~~

~ 18254 same as wt

~ 8582

~ 5000

34589

EXHIBIT

D20

← ~~But it is not like this with EcoRI and NdeI, because you were able to see~~

← It's like this, because NdeI digestion of p21 gave linear form.

⇒ Incomplete digestion? (with Nde)

⇒ cut with EcoRI (pBHG11 as control).
and PacI + BamHI

[redacted]

1) ladder
2) pBHG11/Nde
minipreps: (6ex)
3-26) - clones 1-24

⇒ clones 4, 7, 9, 14, 18, 2[illegible]
→ trash.

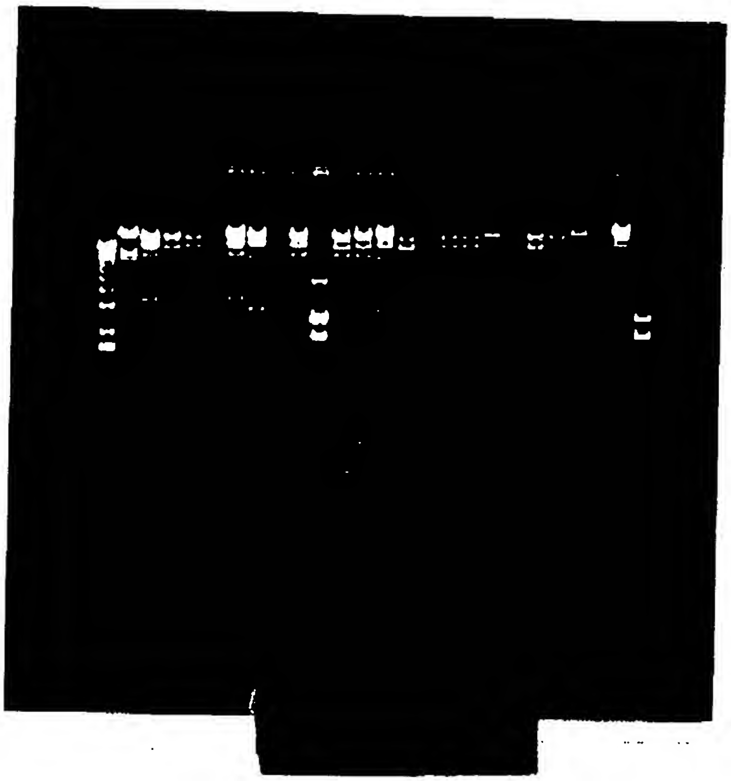
clones 1, 2 and other with same pattern are with correct orientation of insert?!

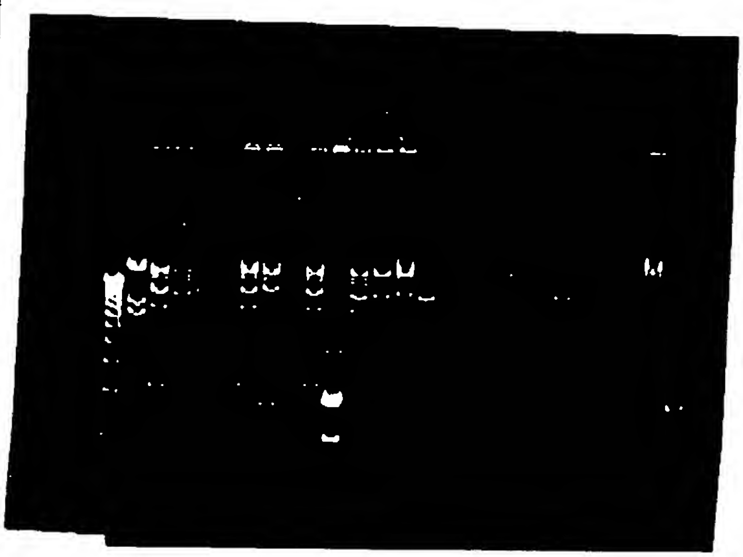
Partial digestion with NdeI!?

Bottom picture is the same.

Or cloning in partially digested pBHG11?
or star activity digested?

take to analize RI
1, 3, 5, 6, 17, 19, 21



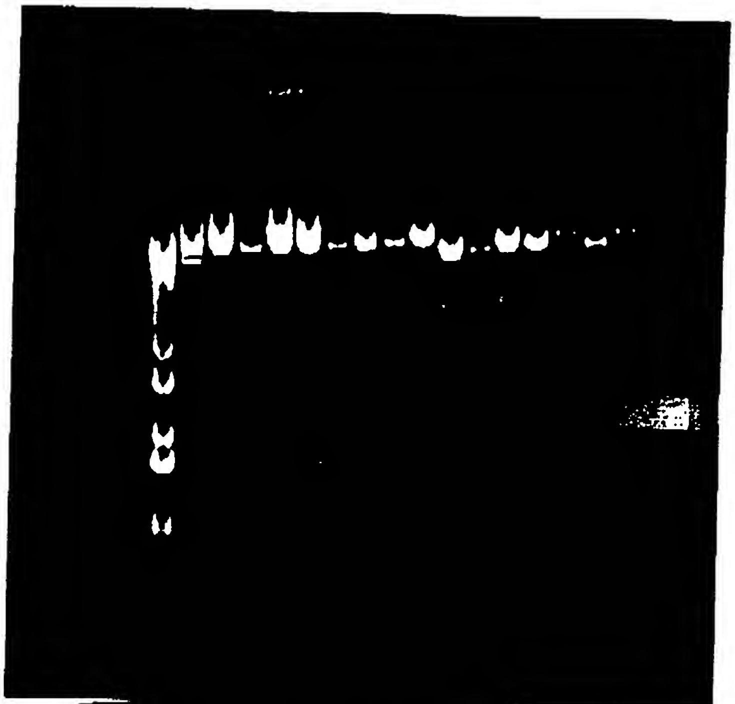


Pac + Bam → NEB buffer 4 - green both enzymes 1:1

~~[illegible]~~

EcoRI - ~~Multicore~~ Buffer 4, Promega

[redacted]



[redacted]

1) Ladder
2) pBUC11/RI
3) 1
4) 3
5) 5
6) 6
7) 17
8) 18
9) 21

RI

10) BUC / Bam + Pac
11) 1
12) 3
13) 5
14) 6
15) 17
16) 19
17) 21
18

Bam + Pac

Conclusions: 17,21 are OK by EcoRI digestion
others are result of EcoRI star activity when preparing vector.

Pac + Bam → pBUC11 gave more fragments than expected
all clones are similar and different from pBUC11 wrong orientation?
they have different orientation,
Pac did not work in clones?

17,21 are not OK inserts are too small !!!???

EXHIBIT
D22

2 orientations

10000	11000.
8000.	7500
5000	5000.
3000	2700

you can explain if just in the case if larger fragments (7+8) are result of partial hydrolysis

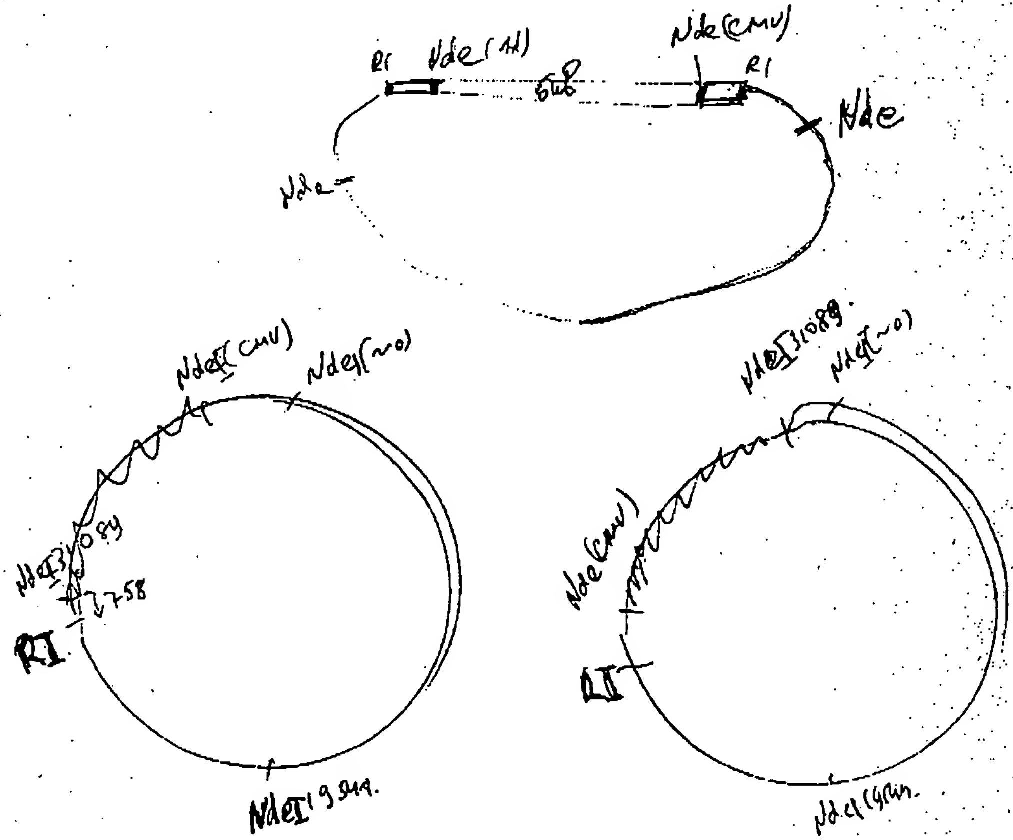


EXHIBIT
D23

If it is mixture of 2 plasmids

and ⇒ there ~~are~~ just 2 Nde sites ?

EXHIBIT

D24

Wednesday, November 20, 1996. Thursday 21 November

1) Seed L1 for mini (midi) prep. with Wizard!
2) Cut TP-DNA Ad5 with EcoRI, check on E/F, dialize against TE.
3) Precipitate Ad5 DNA, check amount, cut EcoRI, phenol, precipitate
4) Split 293 to dishes for transfection.
5) Sequence clones.
6) Prepare new vector pBHGH/RI/CIP ligate with 123/RI insert
7) Seed 17,21 clones for further characterization extract with Wizard kit!
8) Cut pBHGH with RI, Nde, Bam, Pac, Bam + Pac? only with Pac
9) Check SrfI site in pFG140, pBHGH, pXL, some other?

Friday ~~Thu~~, November 22, 1996.

1) Transfection of 293 with pL2 + TPDNA Ad5/RI or ~~DNA Ad5~~/RI
2) Extract 17,21 and L1 with Wizard miniprep.
3) Sequence clones.

seq ~~#~~2 3ex, clone 1 (31) - primer KD2.
seq #3,4 2ex, clone 23 (223) - primers KD10, KD11
seq #5,6 1ex, clone 23 (125) - primers KD10, KD11
seq #7,8 5ex, clone 4 (54) - primers KD6, KD7.

8 µl premix
5 µl template (0.5 γ)
1 µl primer
6 µl H_2O
40 µl oil

circling

Monday, November 25, 1996.

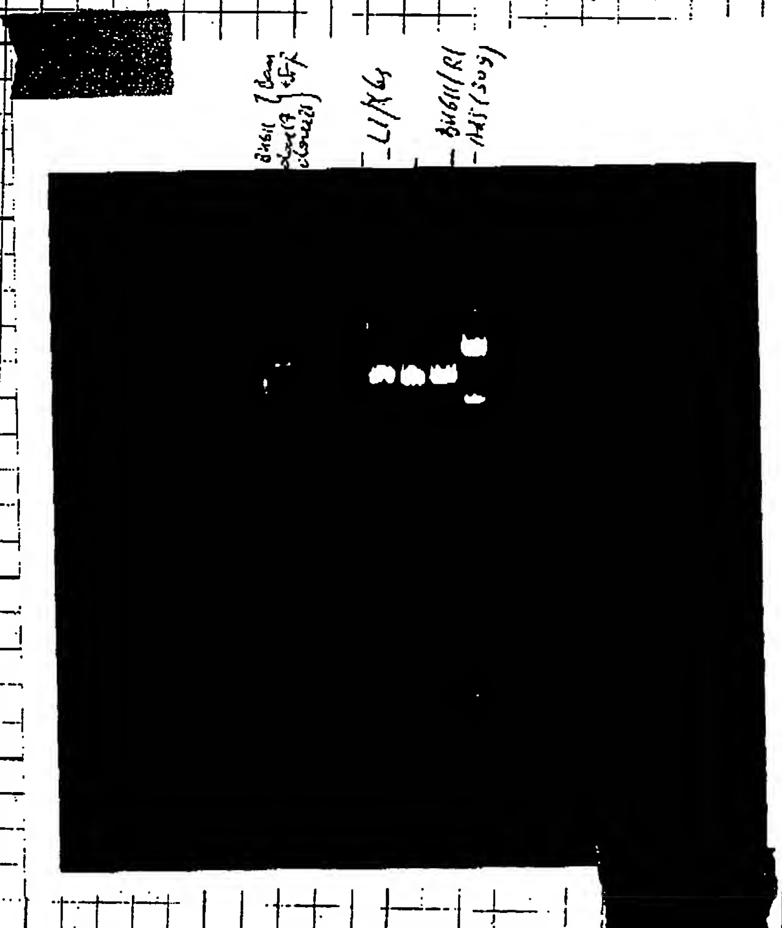
1) Cut new preparation of L1 with Xho, check on E/F.
2) CIP pBAG11/RI.
3) Check amount of AHS/RI.
4) Cut 17, 21 with Bam + Srf, pBAG11 - control → in fragment 5838
5) Check plasmids with SrfI.

SpeI - site → 250 in CMV promoter.

⇒ in right orientation ⇒ fragment ~ 5800 bp

⇒ in wrong orientation fragment ~ 1000 bp.

cut - linear form.



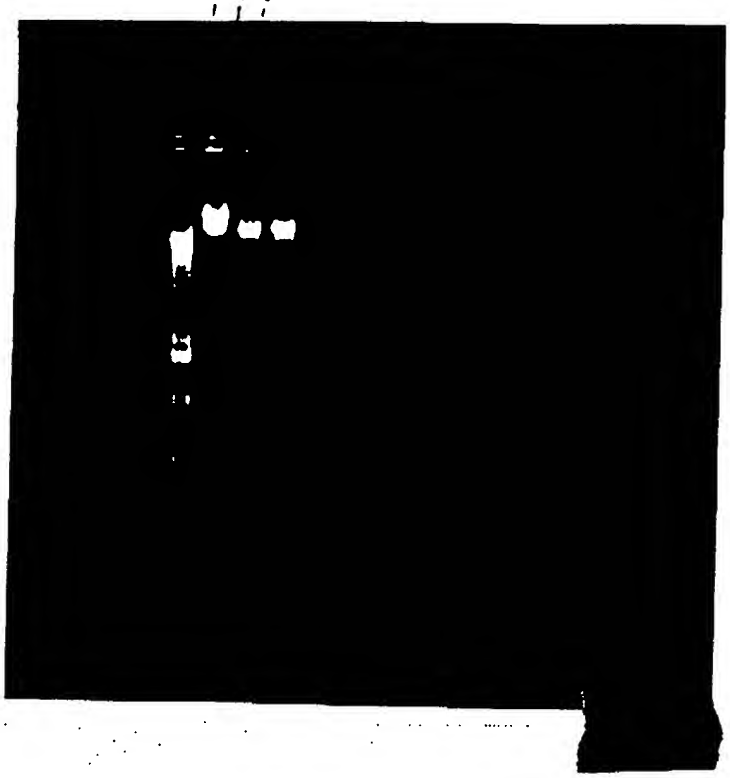
1) Ladder
2) BAG11 } Bam + Srf.
3) clone 17 }
4) clone 21 }
5) L1 / Xho - D } Promega buffers
6) L1 / Xho - C }
7) L1 / Xho - M }
8) BAG11/RI (buffer H)
9) AHS (AZ 309 ?)

Bam + Srf hydrolysis (buffer Multi) strange picture, same as before Bam + Pac ⇒ m. b. 2 Bam sites or star activity?

EXHIBIT
D26

[redacted]

- pUG119
- clone17
- clone21
SpeI



1) ladder

2) pUG11 ⎫
3) clone 17 ⎬ Spe I
4) clone 21 ⎭

there are no fragments
~ 6000 bp ⇒
⇒ wrong orientation
or what ?
⇒ repeat cloning.

Tuesday, November 27, 1996.

1) pBG011/R1/CIP → extract from gel
L1/Xba → CIP → extract from gel
ligate to inserts o/n
3(PCR)/Xba → extract from gel (2%)

1) pBG011/R1/CIP (new prep, 0.1/1) → 10 µl
123 insert (old prep 0.05/5) → 7 µl
2 µl buffer
1 µl enzyme
O/N 16°C

11) pL1/Xba/CIP (new prep, 0.1/5) → 12 µl
3(PCR)/Xba insert (new prep 0.1/5) → 5 µl
→ 2 µl buffer
→ 1 µl enzyme
O/N 16°C

Wednesday, 28, 1996

1) Transform DH5α cells with ligation mixtures from yesterday try to obtain high efficiency!
plate cells in duplicates, 1/10, and all.

2) Plan scheme of experiments to obtain larger plasmids, check everything, start clonings.

3) Split 293 in flask.

Friday 30, 1996.

1) Seed clones 6ex, 4ex in liquid culture and agar plates.

2) Split 293 cells, overlay 293 dishes with transfection!

EXHIBIT
D28

Saturday, November 30, 1996.

1) Extract 6ex 25-36 with Wizard kit, cut with SpeI. Extract 4ex 1-24 with boiling, cut with Sac II.

2) 2) from yesterday.

Sac II - buffer C / or ...

SpeI - NEB buffer II

3) Check references about splicing in Ad, especially in ES and fiber!

4) Start with genes IL and IIc?

* Filter HEBS and $CaCl_2$ from freezer 2 time? !

NB There is no SpeI site in CMV promoter according to my sequence ⇒ may be clones 17 and 21 from previous cloning are OK?

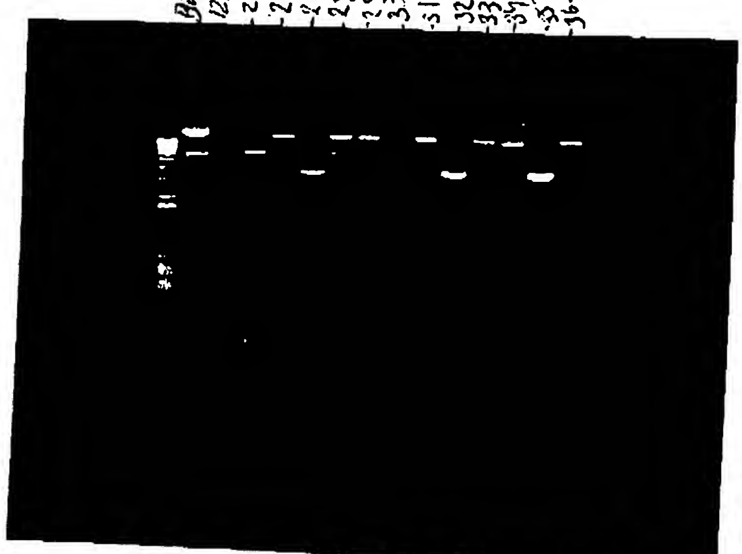
Monday, December 2, 1996.

1) Check new 6ex clones with EcoRI, select recombinants

2) Check new 4ex clones with XbaI, select recombinants.

[redacted]

Bu6II/RI, 123/RI/Af, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36



1)

1) ladder

2) Bu6II/RI

3) 123/RI → A fr.

4 →) 25 → 36.

Recombinants.

26, 28, 29, 31, 33, 36.

2) Cannot see fragment on XbaI hydrolysis.

[redacted]

EXHIBIT

D29

3) Hex SacII (1 site in ADP), no sites in L1
+ RI (0 sites in ADP), 1 site in L1

expected fragments correct orientation ~1261
wrong orientation ~1561

orange buffer (C)

4) 6ex clones Pac + Bam.

1 h Pac (buffer NEB 1 + BSA)
↓ heat inactivate
↓ + 1x buffer ~~C~~? + Bam → 1 h. → E/E.
(~~E~~)?
(D)?

NEB 1 – 10 mM Tris propane-HCl, 10 mM $MgCl_2$, 1 mM DTT (pH 7.0

BamHI unique NEB buffer. – 150 mM NaCl, 10 mM Tris HCl, 10 mM $MgCl_2$, 1 mM DTT (pH 7.9

buffer D – promega pH 7.9, Tris HCl - 6 mM, $MgCl_2$ - 6 mM, NaCl - 150 mM. pH 7.9

D - yellow,

Buffer C (orange) pH 7.9, 10 mM Tris HCl, 10 mM $MgCl_2$, 50 mM NaCl

Mcore (white) pH 7.8, Tris ~~HCl~~ acetate - 25 mM,

100 mM K acetate, 10 mM ~~Mg~~ acetate.

Take orange 2X ?

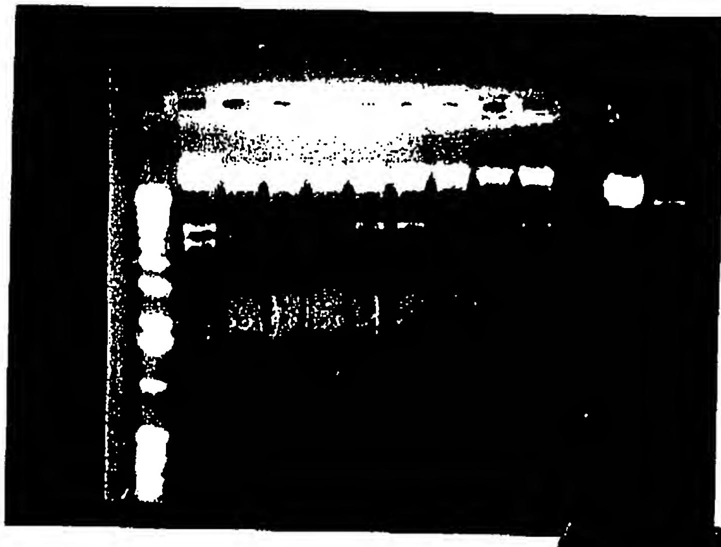
22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS

EXHIBIT
D30

Cloned LD3 ADP
12/2/96

1) ladder
2) pAMG11 / Pac + Bam
3) 17
4) 21
5) 26
6) 28
7) 29
8) 31
9) 33
10) 36
(lanes 3–10: Pac + Bam)
11) blank
12) L1 / Sac II + R1
13) - 24) - clones
4ex (1 - 12) / Sac II + R

the same gel, higher magnitude.



Conclusions:

4ex -
- clones
2, 5, 6, 7, 8, 10 -
- correct orientation
clones 4, 9, 12 -
- wrong orientation

6ex - absolutely unclear what is happening.

yellow ← D

Tomorrow try to cut with Nde I, take cond of plasmid, good activity of Nde I.
Try some other enzymes? (Not Pac, Not Bam)
universal 1X. ← for example SpeI, SrfI.
correct or ⇒ 448

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Deliver to: Dan Kasten

Phone: ______ **Fax:** 552-7305

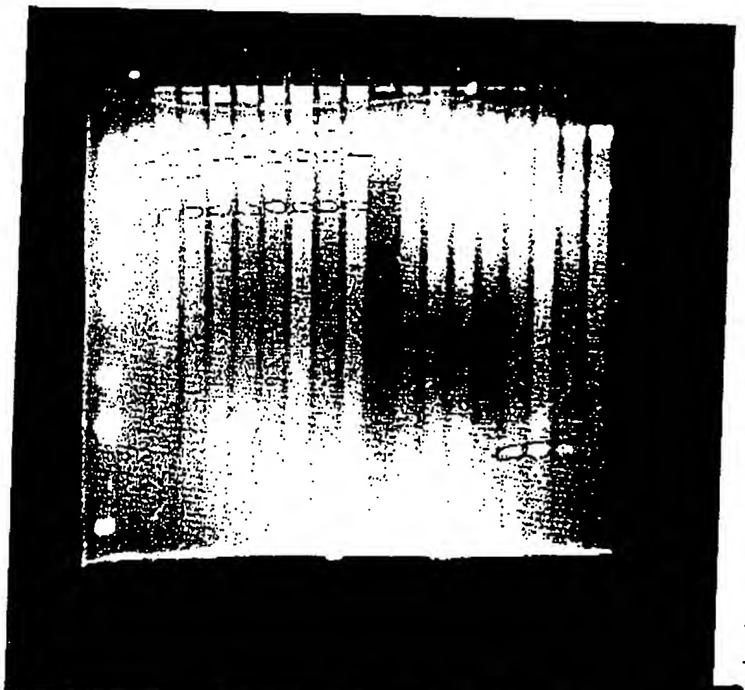
Total number of pages including this page: 18

Message:

Tuesday, November 5, 1996

1) cut 6 ex clones with Nde (Buf. D, yellow)
and SrF + SpeI (buffer universal 1X).

[redacted]



[redacted]

Conclusions: 17, 21, 29, 31, 33 – one orientation
26, 28, 36 – other orientation

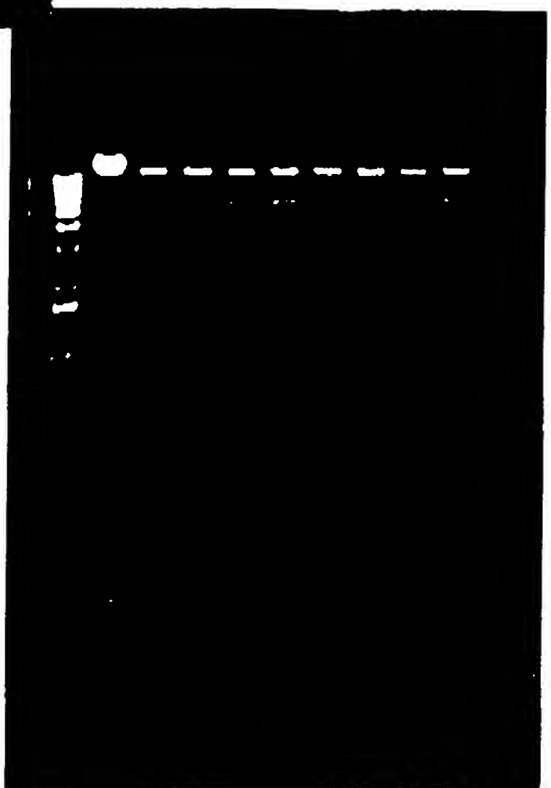
If consider the previous picture, 26 28 36 → correct orientation (smaller fragment because there was additional 60 bp Bam)

Obviously there is SpeI site in CMV + 17, 21 didn't give fragment because they are wrong orientation (on SpeI hydrolisis)

Wednesday, 4, 1996

1) Cut cex clones with SpeI. (buffer Multicore)

[redacted]



[redacted]

1) ladder
2) pBK611 / ~~EcoRI~~, SpeI
3 – 10)
17, 21, 26, 28, 29, 31, 33, 36
– SpeI

there is fragment
6 kb in 26, 28, 36 ⇒
⇒ correct orientation

there is fragment
1200 bp in 29, 31, 33 ⇒
⇒ wrong orientation.

17, 21 – junk! look also to upper fragment on the previous picture.

Conclusion: When everything is OK, it's OK.

Friday, December 6, 1996.

1) Cut pFG140 → BamHI + NdeI fragment 9437 (contains EcoRI site, other fragments do not).
other fragments – ~2500 – if there is no NdeI in pMX2 –
or 1900 6000 if there is NdeI site in pMX2 – 2043

cut 223 (pTi-SPB) with NdeI + BamHI – vector

2) Split 293 cells.
3) ~~[illegible]~~ Reseed clones to a new dish
4) ~~[illegible]~~

Monday, December 9, 1996

1) and 3) 4) from Friday

2) Prepare LB for preparative growing of 6ex
Inoculate LB overnight (15 ml)

3) Prepare tris HCl pH 8,5, 8,0, 7,5, 7,2,
autoclave

Karol → Jane can use 1101/1107 for cotransfection to attempt to obtain a virus with E4 promoter → SP-B promoter substitution.

4) Start cloning EcoRI-Sal fragment pFG140 into pK2 (gp19K*)

pFG140 — BamHI - Nde — buffer D
Sal - EcoRI — buffer D

223 (p21 + SP-B promoter) — Nde - BamHI - buffer D - vector
pK2 (31, gp19K*) — EcoRI - SalI - buffer D - vector.

pFG140 Sal - EcoRI — expected fragments!

if 17869
there is 10585 ← that is what I need
no Sal contains BamHI site!
in pMK2, 6905
379
there is
SalI, then instead of 17869 I will have ~9000 ~8860

Wednesday, December 11, 1996

1) Extract maxiprep of 6ex (clone 26)

2) Extract Wizard 4ex (clones 2, 5)

3) Prepare vectors and fragments from yesterday,
+ 4ex (2) Sal + RI

4) Check 42, 45 with XbaI, RI + SacII (sequence!!)

EXHIBIT
D34

[illegible], 2 [illegible]

[illegible]

[illegible] no [illegible] in prep wx 2,5 ?

223 / BamHI + [illegible] → 1 [illegible] 5000 [illegible] ??
BamHI star activity? M.B. there is 2 site for BamHI ?
It looks like there is 2nd BamHI site
It also [illegible] explain fragment on Bam & something
pBMG " and debilitate [illegible] liso

5) ⇒ Seed [illegible] ([illegible]) and 223 WMP
[illegible] [illegible]

6) Ligation (7 ex) [illegible]

pFG140 Sal + EcoRI → [illegible] (10 kb) (0.1 / 10)
p34 ([illegible]) (3 [illegible]) Sal + RI → A fr (10 kb) (0.1 / 1.0)

7 exper 10 μl vect + 10 μl insert + 2 μl BT + 1 μl ligase
7c 10 μl vect + 10 μl H_2O

Thursday, December 12, 1996

1) Take 626 plasmid from CsCl - EtBr → 2nd spin.
2) Extract 223 WMP (42, 45 - didn't grow) why?
3) Transform the ligation mix from yesterday, 6)
4) Split 293 cells.
5) ~~transform 42, 45 make WMP preps immediately!!~~
Seed 42, 45 again → 60 dish this time!
~~Transform~~ ~~[illegible]~~ 42, 45

Need to 1) Sequence 42, 45 = need WMP
2) Grow up 54 (L2 + ADP) → ready for cotransfection!

EXHIBIT
D35

[redacted]day, December 12+1, 1996.

1) Purify 626 from Cal.[redacted]
2) Check amount of [redacted]-R1 fragment [redacted]
Col-R1 fragment [redacted] are two

Saturday, December 14, [redacted]

1) Prepare LB, sterile eppendorffs, stain [redacted]
Lag[redacted] sterile TE [redacted]
2) Dissolve 626 prep in sterile(!!) TE [redacted] plasmid with [redacted]ases
3) Split 293 cells to [redacted] transfection 626+ left[redacted]
4) Repeat transformation of [redacted] old prep. of [redacted] cells, plasmid,
take with new [redacted] Lag[redacted] dishes(!) 7ex, 7c
5) Transform DH5α (new prep) with 42,45 [redacted]

7ex, 7c ⇒ 10 μl of each to 100 μl of old DH5α cells
42,45 ⇒ 0,001 γ of each to 100 μl of new DH5α cells

Monday, December 16, 1996

7ex, 7c ⇒ no clones again, efficiency of transformation pretty high
What's wrong? ($10^4 \times 1.0^3 \approx 10^7/1\mu g$ pL1 ⇒ ~$10^8/\mu g$ pUC)
Ligation?
Not optimal ratio vector/insert (?)
pBSSK was pBSKS(?)

1) Dissolve 626 [illegible] in sterile TE, check on E/E amount and quality of the plasmid, check other plasmid as well (left arm)
2) Transfection of 293* with 626+ leftarm.
3) Ligate 7ex again, transform DH5α, 5 μl vector, 15 μl insert; transform CMV/TTF1.
4) Seed 42,45 to liquide culture (LB).

pAAV - left arm plasmid (1.5γ/μl) 7μl (10γ)
p662 - (pBUG11 E4del CMV) (0,5γ/μl) 100μl (50γ)

✓ 1,5 ml 2X HEBS
✓ 1250 μl H_2O.
✓ DNA (107μl)
✓ 150 μl $CaCl_2$.
✓ 3ml → for 6 dishes

2X Overlay
for 100 ml overlay

50 ml 2X DMEM
5 ml 7,5% $NaHCO_3$
2 ml ~~FCS~~ ~~100X~~ PS
4.3 ml 1.8% Noble agar.

Tuesday, December 17, 1996.

7ex → no clones. Something in fragment preps. interfere with ligation?. (It is possible, because of this red colour stripe!)

1) Seed i) CMV/TTE, 54 (42+ADP) into large scale liquide culture LB.
ii) TK, 31 → small scale liquide culture → WMP.

2) Extract 42, 45 with Wizard mini prep, check, seed 42, 45 for medium scale prep.

3) Try precipitate fragments 140/SR, 31 SR to purify them.

repeat preparation of this fragments.

4) Try partial digestion p223 Bam Nde (Nde - complete, BamHI - partial dephosphorilate, try cloning with 140 BN fragment.)

Try to clean them with Wizard clean up system.

B

EXHIBIT
D37

Wednesday, December 18, 1996.
Thursday, December 19, 1996

Cut pFG140 → (Bam + Nde (buffer D))
→ SalI + EcoRI (buffer D))

Cut 223 with Nde (complete) → then with BamHI (partial) →
→ blend → CIP → vector from gel.

Cut 31 with Sal + EcoRI (buffer D)).
Cut 293 EcoRI (buffer D)).

31/RI/Sal, 140/RI/Sal, 223 Nde, 223 RI, 140/Bam Nde

Sunday, December 22, 1996.

1) Overlay dishes 293 with neutral red.
Split 293 cells to dishes ⇒ repeat transfection.
Seed frozen 293 cells for low passage.
2) Check 31, 31/Sal, 31/RI, 31 Sal + RI to estimate molecular weight of the plasmid, 223/Nde, and partial with BamHI. 626
3) Cut fragments from gel, ligate.
4) Seed (CMV/TTF), (54) for large scale liquid culture LB.
5) Seed (tk) plasmid for WMP.

Sunday, December 29, 1996.

1) Check i) CMV/TTA - with BglII, SacI.
ii) ptk ? → HindIII + XhoI, PstI.
iii) p54 (prep) - PacI
iv) 42 - XbaI
45 - XbaI
} 2% agarose

EXHIBIT
D38

~~2) Split 293 cells to dishes for transfection~~

3) Cut 1101/1107 DNA with EcoRI.

First attempt to make KO1 12/04/96 (?)

Transfection:

→ 2 dishes pFG1ws.
→ 2 dishes E1 + pBHG11
→ 2 dishes E1 + pBHG11 del E4pr CMVprom ins.

500 μl 2x HEBS + 450 μl H_2O + 20 μl pFG1ws. / 1 + 20 μl BHG11 / 1 + 20 μl BHG11 (E4→CMV) + 50 μl $CaCl_2$

Precipitate is coarse again where E1 plasmid is used ⇒ purify it (SCl banding)

Saturday, December 4, 1997.

1) Cut 1101/1107 with EcoRI.
2) Split 293 to dishes, cotransfect 1101/1107 with L2, 54,
3) Pick up this ugly things resembling plaques + to plate to try to grow up a plaques
4) Overlay dishes with NR.

Overlay 2X 200 ml

200 ml 2x DMEM
4 ml 2x PS
2 ml 5% yeast extract.
3 ml FBS
10 ml HS
2 ml 100x L-Glutamine
20 ml bicarb 7.5%.

Transfection. 1101 / 1107 /RI + ~~control (H_2O)~~
2(γ) + L2 (20γ)
+ 54 (20γ)

4 ml of each transfection mixture

2 ml of 2x HEBS.
1.8 ml of H_2O
DNA
+200 μl $CaCl_2$ 2.5 M.

Wednesday, 8, 1996.

1) Spin left hand plasmid in ~~[illegible]~~ gradient 2X time.
2) Split 293 to dishes for transfection.
3) Cut 31 with EcoRI → complete digestion and with Sal I → ~~fragment~~ dephosphorylate.
4) Cut 223 (21 + SPB prom) / Nde with BamHI partial → dephosph. → fragment from gel → Vector.
5) ~~Fragments~~ Fragments (140 Sal + Nde, Bam + Nde → cut from gel,
6) Ligate. 7ex, 16° overnight.
7) Precipitate 1101/1107 DNA 2nd time, cut with EcoRI!
8) Sequence 42.
9) Plan experiment with TK gene.

Thursday 9, 1996
1) Transform Kurt's Left-hand plasmid

Monday, 14, 1996

Transfection 5γ lacZ (5μl) + 15γ 626 (15μl) (1:3)
or 10γ lacZ (10μl) + 10γ 626 (10μl) (1:1)
or 5γ lacZ (5μl) + 20γ 626 (20μl) (1:4)

Ligation 8ex 225 / Nde + Bam (partial) C, P
+ 140 / Bam Nde B fragment

Thursday, 17, 1996.
1) Sequence 42, primers (21) 4, (21) 5 →
→ sequence → (21) 9, (21) 10:
→ No sequence at all!
What is it?

EXHIBIT
D40

8 µl premix
3 µl p42
7 µl primer (4 or 5)
8 µl H_2O

circ. lig.

Monday, 20, 1886.

8ex (p223 / complete hydr. Nde + Partial Bam)
(+ B fragment pFG(40 / Bam + Nde.)

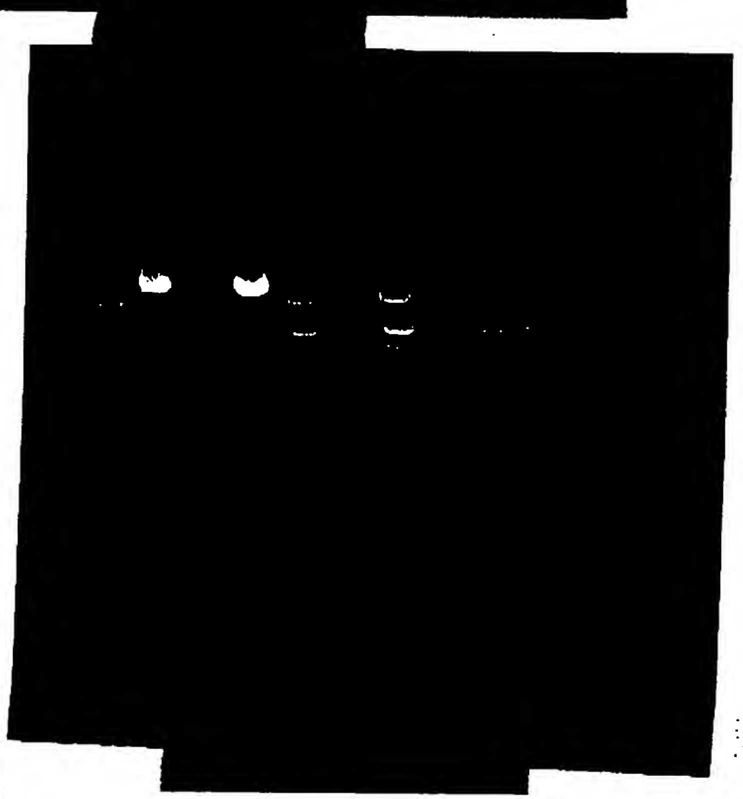
[redacted]

Minipreps: 8ex (1-12).

1) ladder.
2) p223.
3) →
clones 8ex 1-12.

1) ladder
2) pFG140 / Bam + Nde
3) p223 Bam + Nde.
(upper fragment – because of incomplete Nde digestion)
4) 81
5) 82
6) 86
7) 87
8) 88
9) 89
10) 816

Nde + + Bam



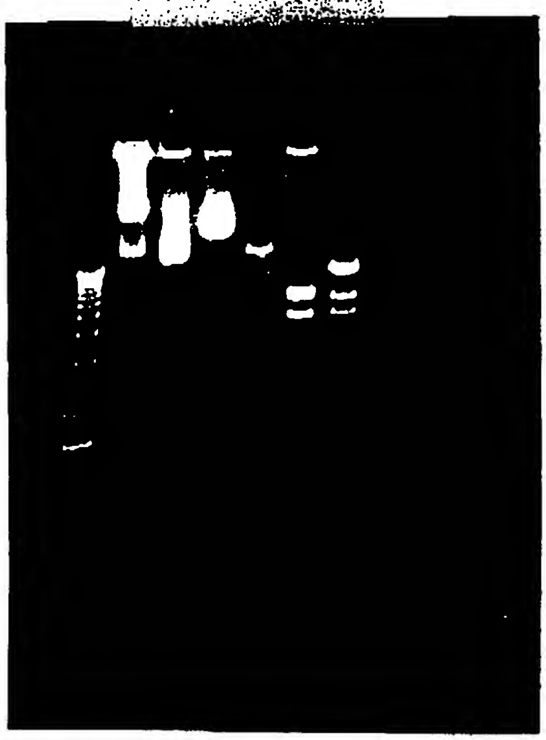
clones 82, 86, 87,

Cloning 9ex ⇒ 82 / ~~EcoRI~~ + Xba I – vector A fragment ~ 10 kb
B fragment ~ 9 kb

insert p81 / EcoRI + Xba – A fragment
~~[illegible] / NotI EcoRI + Xba~~
~~NdeI – Xba I~~

Ligation vector 82/RI Xba A fr. 5 µl (0.1/10)
insert 31/RI Xba A fr 5 µl (0.1/15)
7 µl H_2O
+
2 µl buffer
1 µl ligase

2 x CsCl prep. of 82
1) ladder
2) 240
3) 823
4) 82
5) 410
6) 823
7) 82
Bam + NdeI.



January ~~25, 1994~~.

9 c and 9ex → ~100 bact. colonies / dish in both cases / ⇒ do fresh plasmid vector repeat trafo.

Transfection: 1101/1107/EcoRI (0.5γ/μl)
+ p82 (E4→SPB) (1γ/μl)
+ CMV/TTF (2γ/μl)

10 6 cm dishes: 4 ~~control~~ + 6 experiment

1) control A) 8 μl (4γ) 1101/1107/EcoRI (1 ml precip)

2.) control B) 8 μl 1101/1107/EcoRI (4γ) + 5 μl CMV/TTF (10γ) ⇒
⇒ ~~control~~ on wt and toxicity of ~~TTF~~.

3) exp. 8 μl (1101/1107/EcoRI) ⇒ 4γ

A) + 10 μl p82 → 10γ.
+ 3 μl CMV/TTF ⇒ 6γ

B) 8 μl 1101/1107/EcoRI ⇒ 4γ,
+ 5.0 μl p82 ⇒ 5γ
+ 5 μl CMV/TTF ⇒ 10γ

c) 8 μl 1101/1107/RI ⇒ 4γ
+ 15 μl p82 ⇒ 15γ
+ 1 μl CMV/TTF ⇒ 2γ

EXHIBIT
D43

Sunday, February 2, 1987

Transfection

8 large dishes.

2 - 626 + lac z (left-hand)

2 - 11 + lac z (left-hand)

2 - 1101/1107/RT - control + CMV/TTF

2 - 1101/1107/RT + p82 + CMV/TTF

40γ DNA for 2 ml of precipitate - each dish

626 - 4 ml of precipitate ⇒

⇒ 2 ml HEBS 2X. + 100 μl $CaCl_2$

p626 = 80 μl (40γ) ✓ 2 ml H_2O.

placZ = 40 μl (40γ) ✓

11

p11 = 40μl (40γ) ✓

placZ = 40μl (40γ) ✓

(82 - control) ✓ ~~pM~~ 1101/1107/RT - 25μl (12γ)

✓ pCMV/TTF - 20μl (40γ)

82 - exper.

✓ 1101/1107/RT - 25μl (12γ)

✓ pCMV/TTF - 20μl (40γ)

✓ p82 - 30μl (40γ)

EXHIBIT

D44

Wednesday, February 5, 1997

Transfection of 293 with 626 + Lac Z (Mohan's with ITR)
~~811~~ + lac Z (— " —)
626 + lac Z (Good's pAV54...).

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

626 + M
2 ml HEBS 2X + ~~2 ml~~ H_2O + 100 µl $CaCl_2$.
√ p626 – ~~100~~ µl (~~50~~)
√ pMohan's – 5 µl (8 γ)

811 + M
√ pDUG11 – 50 µl (50 γ)
√ pMohan's – 5 µl (8 γ)

626 + C
√ p626 – 100 µl (50 γ)
√ pAV53 – 20 µl (20 γ)

TTC $\boxed{\text{TCTAGA}}$ AGT.... ATGA(GA$\boxed{\text{TC}}$)TAGA

1) ladder
2) - 42/Xb
3) - 45/Xb

⇒ linear form

2nd Xb in the plasmid is methylated!

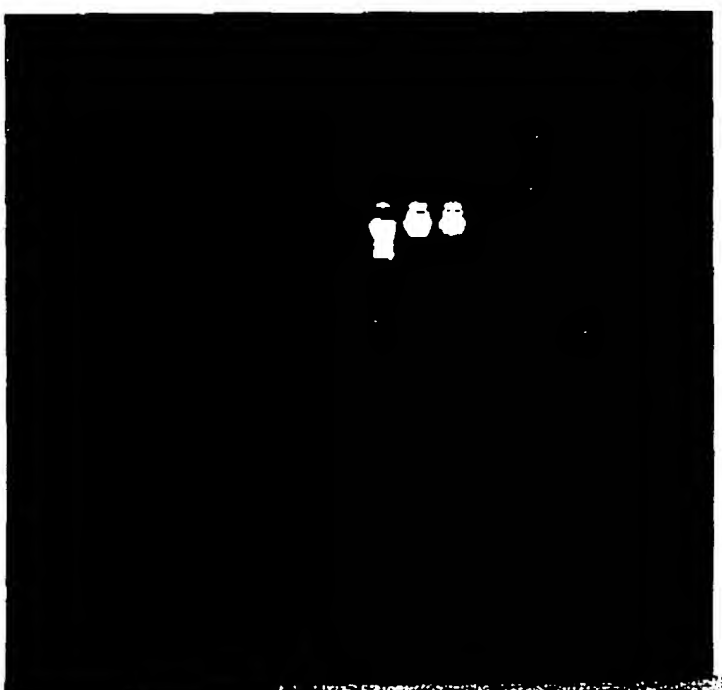
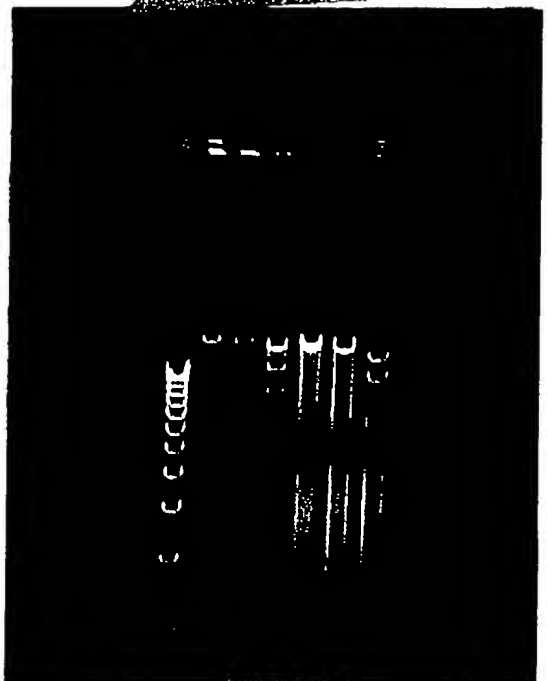


EXHIBIT
tabbies
D45

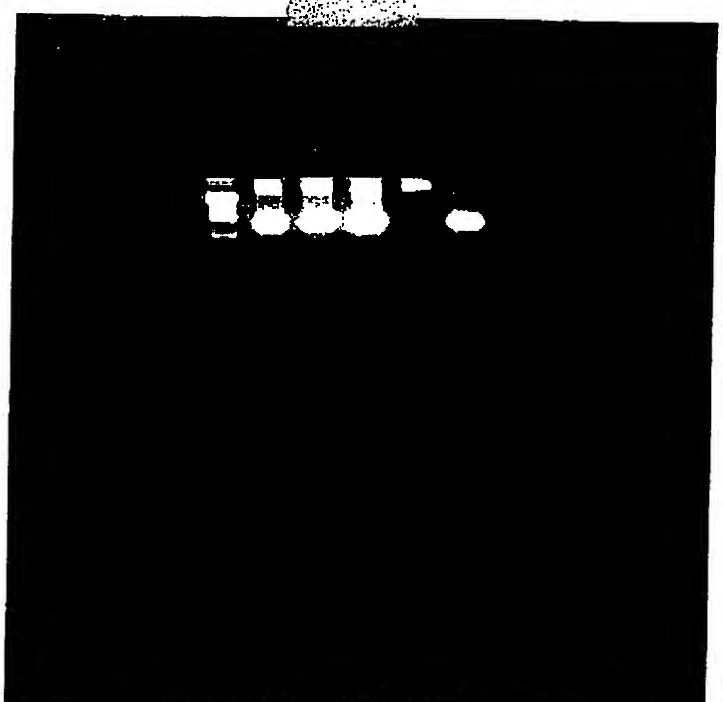
Sunday, February 9, 1997



Analysis of 1101/1107 (R/+
54
plaques isolate 54-2.

1) Ladder.
2)
3) 1101/1107 Pac I
4) Pac I + BamHI
5) 54-2
6) 54-2 Pac
7) 54-2 Pac + BamHI

Conclusion 54-2 – wt
but there are
additional bands



PCR, primers
KD 1, KD 5

1) ladder
2) pEG140
3) p82
4) p54
5) plaque 54-2

Conclusion –

54-2 ⇒ wt E3.

February 10, 1997.

Transfection of Malia's 293/CMV/TTF with p82.

Control : 50 μl of 1101/1107/EcoRI (10γ)
+ 20 μl pCMV/TTF (20γ)
+ 100 μl p82 (100γ)

3 ml H_2O
3 ml HEBS 2X
300 μl $CaCl_2$ 2.5 M.

p82
50 μl 1101/1107/RI.
+20 μl pCMV/TTF
+ 100 μl carrier DNA

February 11, 1997.

Repetition of 19ex. vector → CIP → Heat inactivation.

82/RI+Kle (vector) – 5 μl –
81/RI+Kle (insert) – 10 μl
Lig. buffer – 2 μl
ligase – 1 μl.

February 14, 1997.

Transfection with 1101/1107/EcoRI + or pL2 / p54 (L2+AAP).

Cont A 10 μl 1101/1107/EcoRI (5γ) + nothing
Cont B —"— + 30 μl carrier (30γ)
L2 A —"— + 30 μl L2 (30γ)
L2 B —"— + 30 μl L2 (30γ)
54 A —"— + 50 μl 54 (25γ)
54 B —"— + 50 μl 54 (25γ)

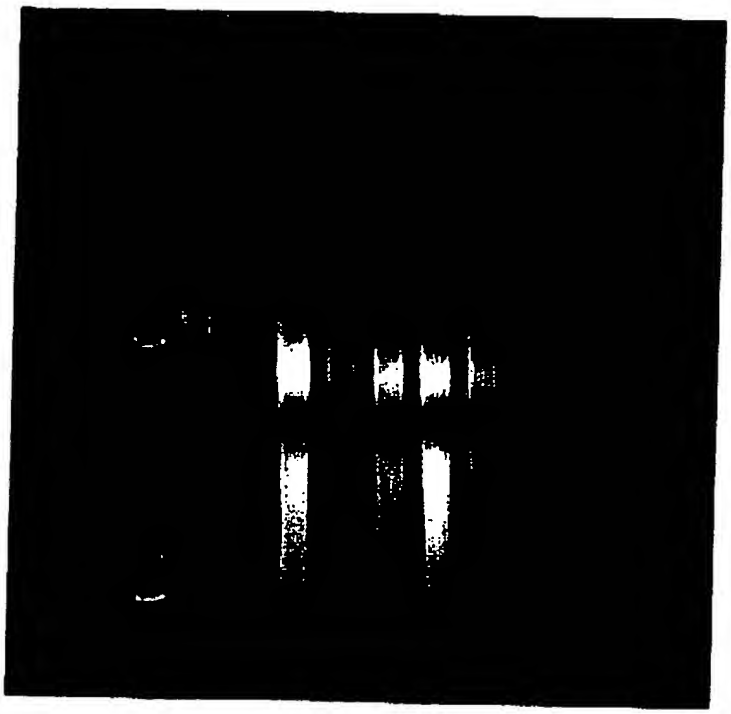
1 ml HEBS 2X + 1 ml H_2O + 100 μl $CaCl_2$.

Cloning experiment 9 (9ex)
with new vectors 82/Ula + R1 (1)
82/R1 + Ula (2)

C vect 0.1/5 Bite ~ 9?
C insert 0.1/5 ~ ~ 9?

9ex 1 – 5μl vect + 5μl insert
9ex 2 – 5μl vect + 5μl insert

February 20, Thursday



Analysis of restriction pattern of plaques:

1) ladder 1kb
2) 110/11-07 / PacI + BamHI
3) mock
4) 54-1
5) 54-2
6) 54-3
7) 54-4
8) 54-5

PacI + BamHI

for 54-1
54-3
54-4
restriction pattern is as expected for recombinant virus!

EXHIBIT
D48

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Health Sciences Center
School of Medicine

Facsimile Transmittal

Date: 12-3-02

From: William Wold

Phone: 314-577-8432 **Fax:** 314-773-3403

Deliver to: Dan Kasten

Phone: ______ **Fax:** 552-~~7~~6305

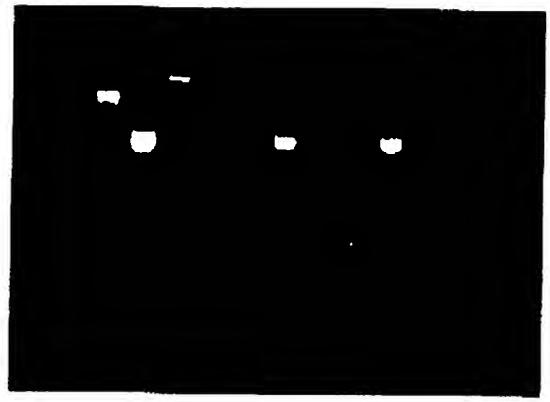
Total number of pages including this page: 12

Message:

Kostya's lab notes.

KO1 is made
2/20/97

2-20-97.



PCR of kint pr
from previous picture
primers: KD 1 and KD 7

1) ladder 1 ul.
2) pFG140 (dl 309)
3) p54
4) mock
5-9) -
- 54 1-5
respectively.

viruses 54-2
and 54-5
contain both wt
and recombinant ADP;

there is band as in wt in mock!

54-1
54-3
54-4
} - recombinants!

02-25-97

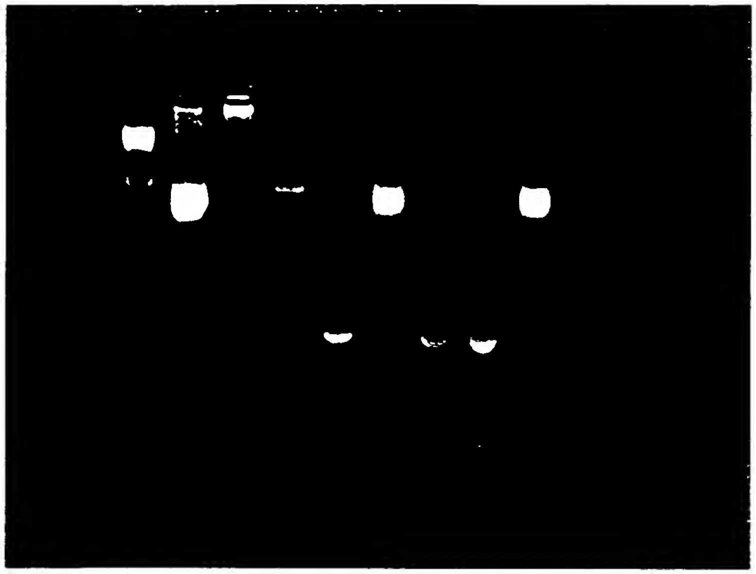
9ex. → 2 time purified thru gel vector.
p82 / RI Xba
insert p31 / RI Xba } A fr.

vector 10 μl 82 + 8 μl H_2O + 2 μl buffer

vector+lig 10 μl 82 + 8 μl H_2O + 2 μl buffer + 1 μl ligase

vector+insert 10 μl 82 + 8 μl insert + 2 μl buffer + 1 μl ligase

2-20-97



PCR of Hirt preps
from previous picture.
primers LCD 1 and LCD 7.

1) Ladder 1 µl.
2) pFG140 (dl 309)
3) p54
4) Mock
5-9) –
– 54 1 – 5
respectively.

viruses 54-2
and 54-5
contain both wt
and recombinant ADP!

there is band as in wt in mock!

54-1
54-3 } – recombinants!
54-4

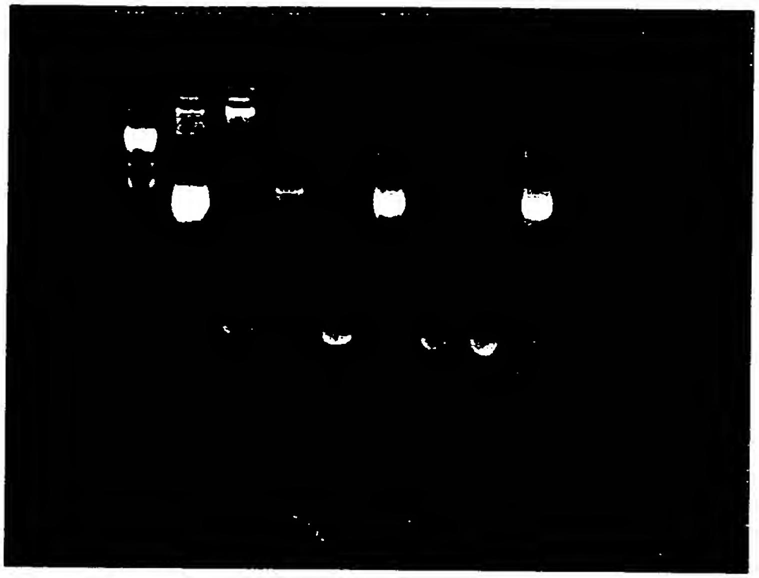
2-25-97

Gel. → 2 time purified from gel vector.
p82 / R1 Xba
insert p31 / R1 Xba } A fr.

vector 10 µl 82 + 8 µl H_2O + 2 µl buffer
vector + lig 10 µl 82 + 8 µl H_2O + 2 µl buffer + 1 µl ligase
vector + insert + ligase 10 µl 82 + 8 µl insert + 2 µl buffer + 1 µl ligase

2-20-97

PCR of Hirt preps from previous picture.
primers LCD 1 and LCD 7.



1) ladder 1 ul.
2) pFG140 (dl 309)
3) p54
4) mock
5-9) — 54 1-5 respectively.

viruses 54-2 and 54-5 contain both wt and recombinant ADP.

there is band as in wt in mock!

54-1
54-3
54-4 } - recombinants!

02-25-97

gel → 2 time purified thru gel vector.
p82 / RI XbaI
insert p31 / RI XbaI } A fr.

vector 10 ul 82 + 8 ul H_2O + 2 ul buffer

vector + lig 10 ul 82 + 8 ul H_2O + 2 ul buffer + 1 ul ligase

vector + insert 10 ul 82 + 8 ul insert + 2 ul buffer + 1 ul ligase
~~+ ligase~~

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS

EXHIBIT
D49

02-25-97
S4-10 plaques - phenotype that S like
wt ~~+ rec~~ or rec with high MOI ?

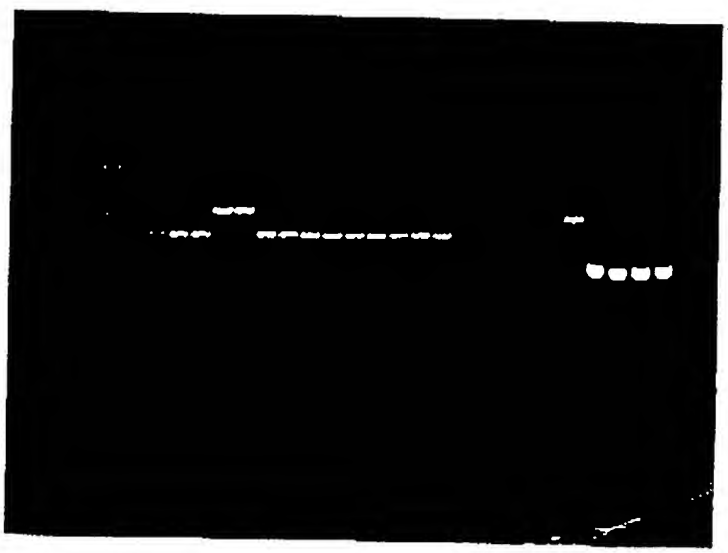
Sex - try Srf 16 ?

03-05-97. Wednesday.

Cloning Experiment 10 ex ⇒ pL2 + E3 from gp19K (p31)
(sites EcoRI + Nde I).

11 ex ⇒ pL2 + E3 from dl E3X6e + ADP (p42)
(sites ⇒ EcoRI + Nde I).

EXHIBIT D50



PCR analysis of plaques p82 and p54

1) Ladder
2) Blank
3) mock
4) 110/1197
5) PFG 140.
6) p225
7) p82
8) wt 110/1197
9) 821
10) 823
11) 824
12) 826
13) 828
14) 829
15) 8210
16) S21

primers (L) 10, (L) 11

17) Ladder
18) blank
19) mock
20) 110/1197
21) pFG 140
22) S4
23) S46
24) S48
25) S49
26) S410.

primers (L) 1, (L) 8

02-25-41

p82 + 1101/1107 / Eco R1 plaques!

AC wt 1 - medium size } wt control
AC wt 2 - large size

transfection from 01-25

A 1, 2 - medium

B 3 - medium
4 - small
5 - medium

B 6, 7, 8 - medium
9 - small

C - 10 - medium
11 - small

12, 13, 14 - medium } plaques ? or not.
15, 16, 17 - medium

transfection from 02-02

Fungal contamination in plate wells

827 and 547 } lost due to brokage of Eppendorf tubes.

⇒ no recombinants with p82 according to PCR results.
p54 = ? ⇒ no product of PCR, just RNA.

05-06-97

Ligation 10 ex, 11 ex

5 µl vector (L2/ENAH) → V

+ ligase → VL

+ 15 µl 31 ENL → 10 ex L

+ 15 µl 31 ENH → 10 ex H

+ 15 µl 42 EN → 11 ex

PCR Analysis of plaques

1) ladder
2) 140
3) 54
4) mock
5) WT 1101/1107
6) 546
7) 548
8) 549
9) 5410

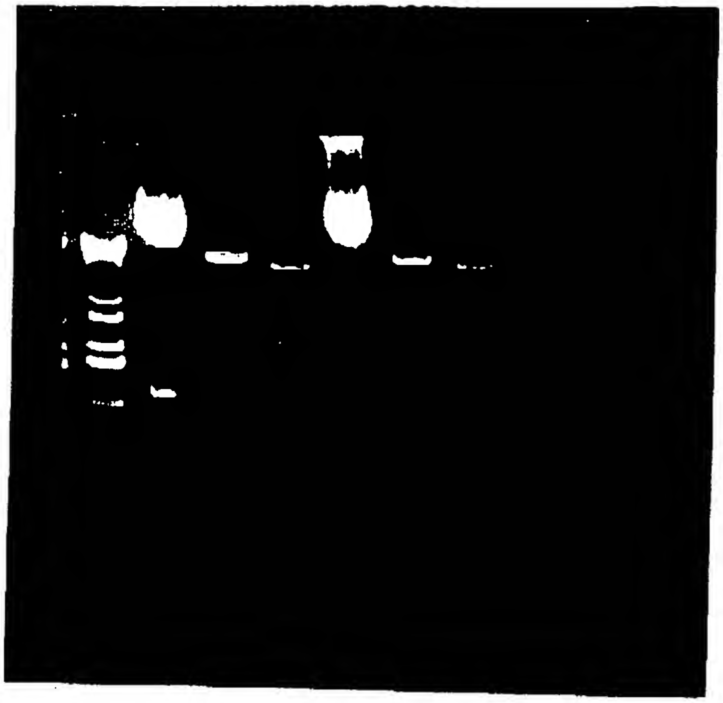
primers w/ 1, 7.

10) ~~blank~~
11) pFG140
12) p82
13) mock
14) WT 1101/1107
15) 822
16) 825
17) 827
18) 829

primers w/ 10, 11.

548, 549, 5410 + recombinants

with p82 all plaques are wt.



1) Ladder
2) pL2
3) p31
4) p42

EcoRI + NdeI, buffer MC

5) pL2
6) p31
7) p42

EcoRI + NdeI, buffer D

03-07-97

Transfection p82 into 293/TTF.

12 dishes — 6 - control (3 ml of precip.) — 60 γ DNA
6 - experiment (— " —)

control 25 μl 1101/1107/R1 (5 γ) 1.5 ml HEBS 2X
\+ 5 μl pCMV TTF (10 γ) 1.5 ml H_2O
\+ 30 μl ~~gal~~ carrier DNA. (~30 γ) 150 μl $CaCl_2$

exper. p82 25 μl viral DNA.
\+ 5 μl pCMV TTF
\+ 30 μl p82

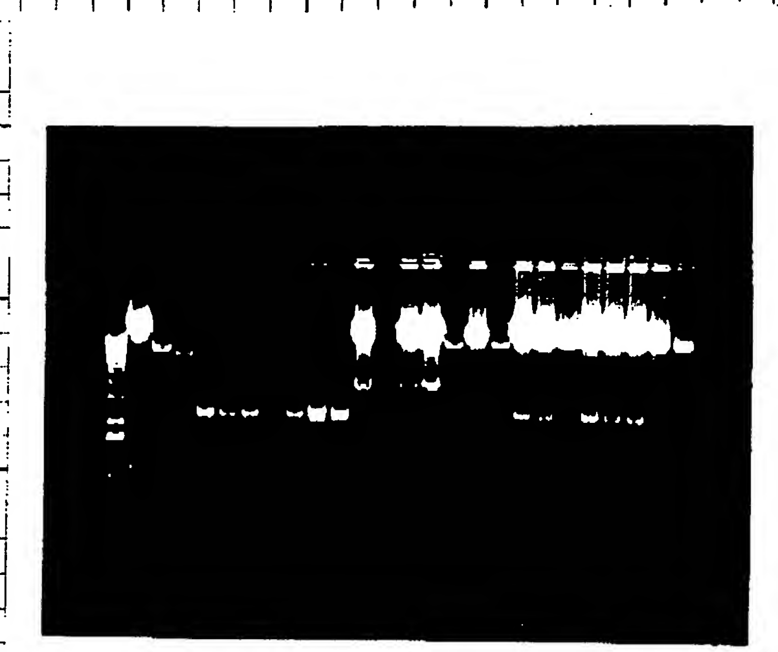
EXHIBIT
D53

03-10-97

Cloning 10ex, 11 ex

1 - ladder
2 - p12
3 - p31
4 - p42
5 - 10L-1
6 - 10L-2
7 - 10L-3
8 - 10L-4
9 - 10L-5
10 - 10L-6
11 - 10L-7
12 - 10H-1
13 - 10H-2
14 - 10H-4
15 - 10H-5
16 - 10H-6
17 - 10H-7
18 - 10H-8
19 - 11-1
20 - 11-2
21 - 11-3
22 - 11-4
23 - 11-5
24 - 11-6
25 - 11-7
26 - 11-8

EcoRI + NdeI



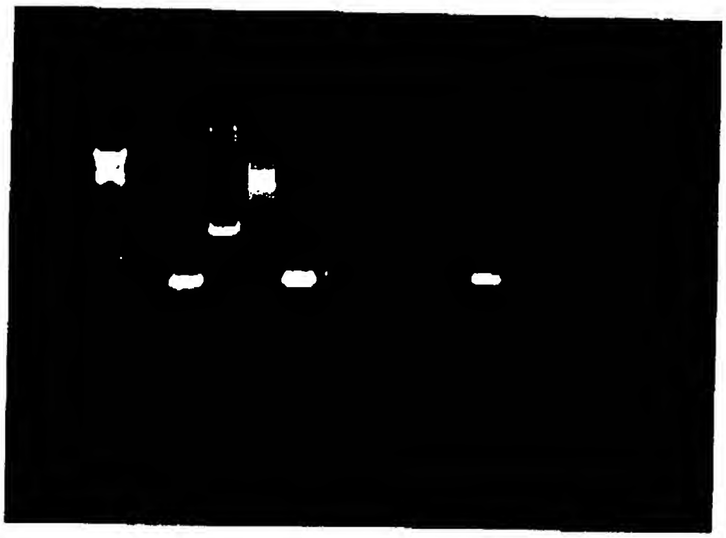
11 - all clones - rect.

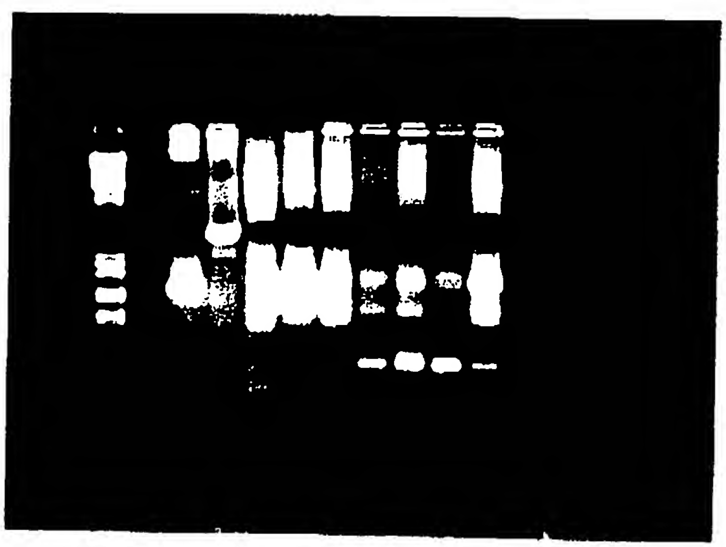
10H - all clones - recombinante
lower band was visible in all clones in short run

take 11 and 10 for CsCl preps.

03-12-97

Hirt preps of small amount (plate) of 293/TTF infected with slow growing viruses p82(?).
PCR, primers 10, 11.





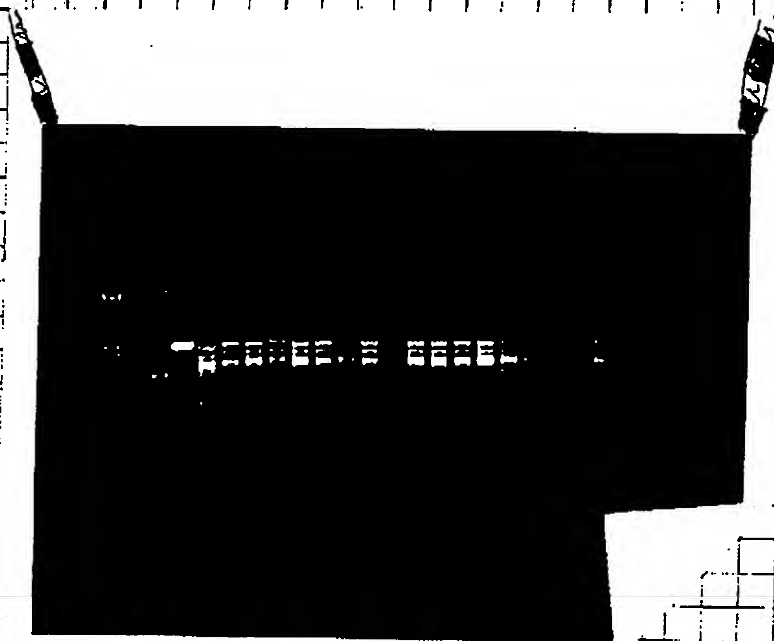
1) ladder.
2) blank (H_2O)
3) pF61ho.
4) p82
5) mock.
6) wt.
7) 8212
8) 8214
9) 8215
10) 82-16
11) 82-17.

Conclusions:
blank - clean.
in 82-12, 82-17 - band, corresponding to wt. and weak additional band corresp. to rec. (does not present in wt!!!)
Something of a bit smaller MW present in mock

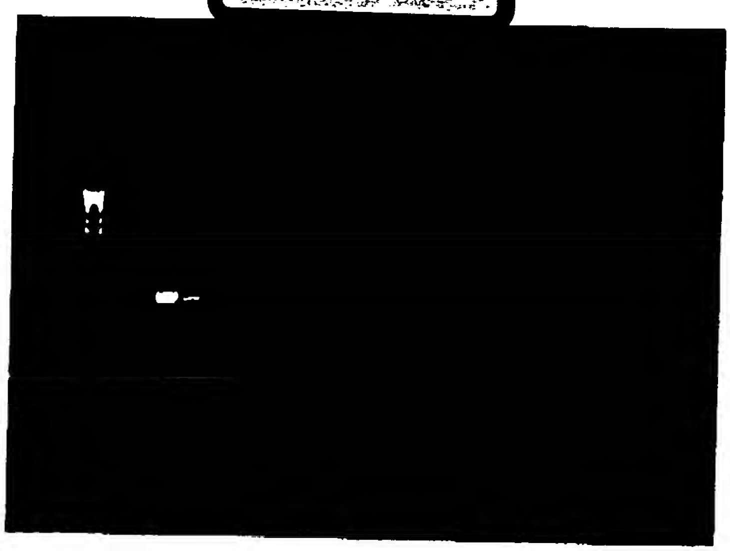
EXHIBIT
D55

3-17-97

PCR analysis of p82 resulting plaques (primers UD 8, UD 9 — SPB - promoter - specific).



1) ladder
2) blank
3) pF6 (40
4) p82
5) mock
6) wt 1.01/1.02
7) 821
8) 822
9) 823
10) 824
11) 825
12) 826
13) 827
14) 828
15) 829
16) 8210
17) 8211
18) 8212
19) 8214
20) 8215
21) 8216
22) 8217



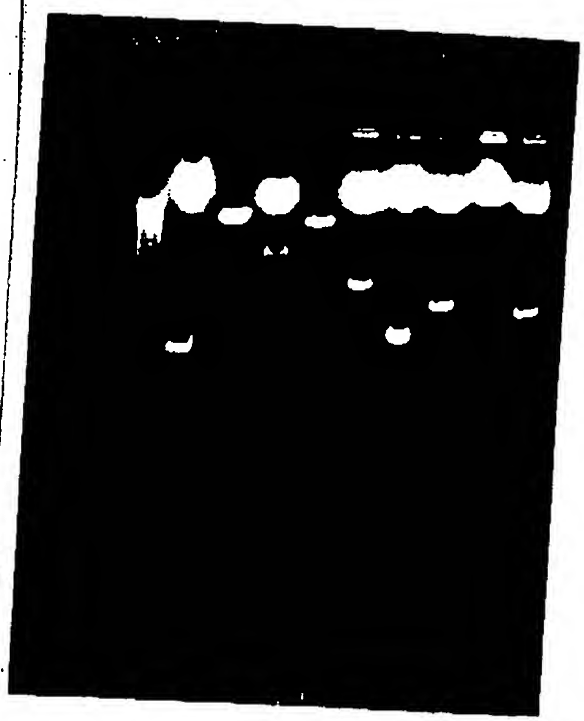
Conclusion: is SPB in 293 cells, viruses are rather wt than recombinants, need to use (UD 9, UD 11) combination of primers / 9 — SPB specific.

3-18-97

Sequencing

KD 9 (p 21 , primer KD 10)
KD 10 (p 21 , primer KD 11)
KD 11 (p111 , primer KD 4)
KD 12 (p111 , primer KD 5)

Restriction pattern of CsCl preps. of p101, p111



1) 1kb Ladder
2) L2 / RI + NdeI
3) p31 / RI + NdeI
4) p101 / RI + NdeI
5) p42 / RI + NdeI
6) p111 / RI + NdeI
7) L2 / PacI + SpeI
8) p101 / PacI + SpeI
9) L2 / SpeI + XbaI
10) p111 / SpeI + XbaI

EXHIBIT D57

3-21-97

Analysis of sequences KD 9, 10, 11, 12.

Everything is fine with p111. As predicted.

p21 - sequence with KD 11 primer is fine.
- sequence with KD 10 primer shown that Tn5 E. coli sequence is going after Ad nt 35858 and up to the end of sequence (~600 bp) → see

⇒ p626 and p82 are not responsible for virus

Need to check pBK611 for the same mutation.

EXHIBIT

D58

Plates KO3
3/27/97

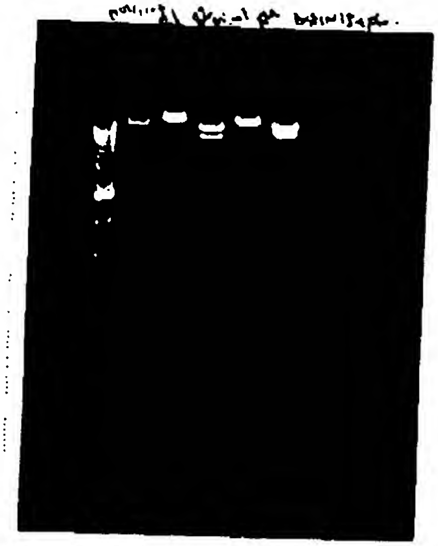
3-20-97 Plaques. (experiments of 02/05 02/02)

626 M 1-12 (12)

626 c 1-6 (6)

11 M 1-4 (4)

3-26-97



1) Ladder 1 kB

2) 1101/1107 DNA / EcoRI + SpeI

3) p82

4) p82 / Bst 1107 I

5) p82 / XbaI

6) p82 / Bst 1107 I + XbaI.

There is Bst 1107 I site in p82 at Ad 29012 !

2nd is after SPB promoter!

3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI
8X 6cm dishes.

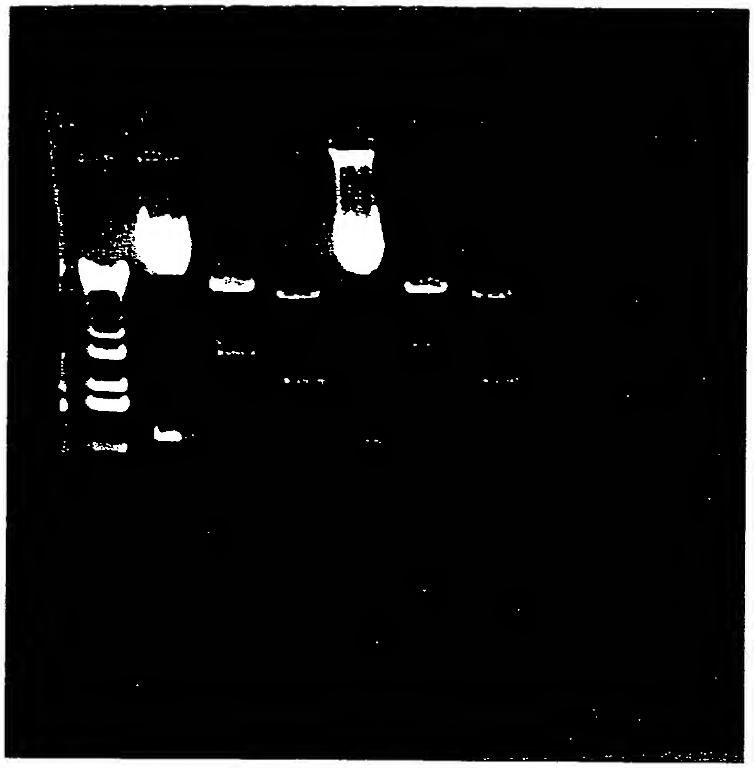
3 dishes 101 (for 1 dish) 250 μl HEBS 2X + 200 μl H_2O + 10 μl 101

3 dishes 111 (for 1 dish) 250 μl HEBS 2X + 200 μl H_2O + 10 μl 111

2 dishes control (for 1 dish) 250 μl HEBS 2X + 200 μl H_2O ——

+ 10 μl 1101/1107 + 25 μl $CaCl_2$.

EXHIBIT
DS9



1) ladder
2) pL2
3) p31
4) p42

EcoRI + Nde, buffer MC

5) pL2
6) p31
7) p42

EcoRI + + Nde, buffer D

03-07-97

Transfection p82 into 293/TTF.

12 dishes — 6 - control (3 ml of precip.) — 60 γ DNA
6 - experiment (— " —) — " —

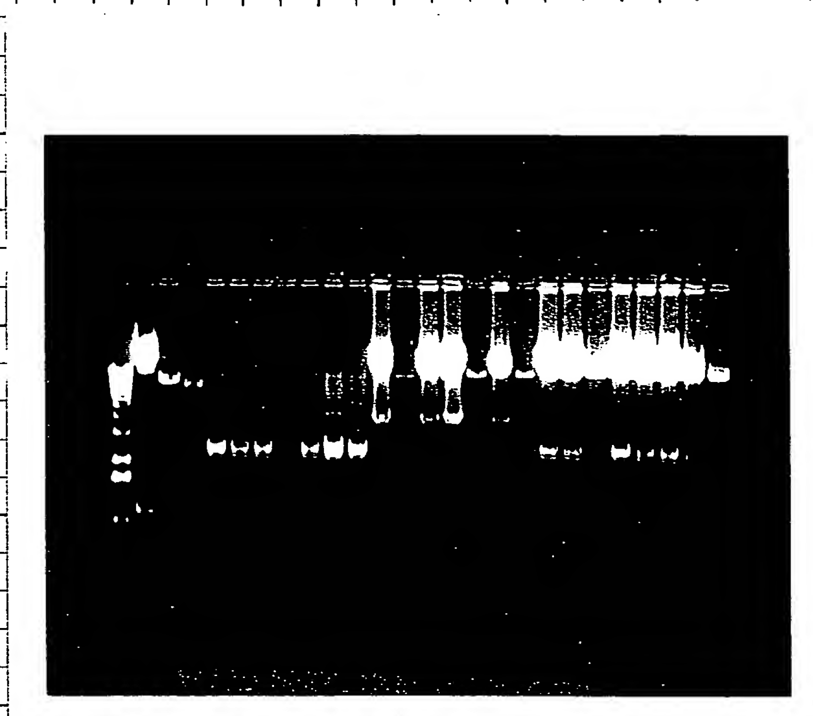
control
25 μl 1101/1107/RI (5 γ) 1.5 ml HEBS 2X
+ 5 μl pCMV TTF (10 γ) 1.5 ml H_2O.
+ 30 μl ~~carrier~~ carrier DNA (~30 γ) 150 μl $CaCl_2$

exper p82
25 μl viral DNA
+ 5 μl pCMV TTF
+ 30 μl p82

03/10-97.

Cloning 10ex, 11 ex.

1 - ladder
2 - p12
3 - p31
4 - p42
5 - 10L-1
6 - 10L-2
7 - 10L-3
8 - 10L-4
9 - 10L-5
10 - 10L-6
11 - 10L-7
12 - 10H-1
13 - 10H-2
14 - 10H-4
15 - 10H-5
16 - 10H-6
17 - 10H-7
18 - 10H-8
19 - 11-1
20 - 11-2
21 - 11-3
22 - 11-4
23 - 11-5
24 - 11-6
25 - 11-7
26 - 11-8



11 - all clones - rect'r.

10H - all clones - recombinate

lower band was visible in all clones in short run.

take 11 and 10 for CsCl preps.

EcoRI + NdeI

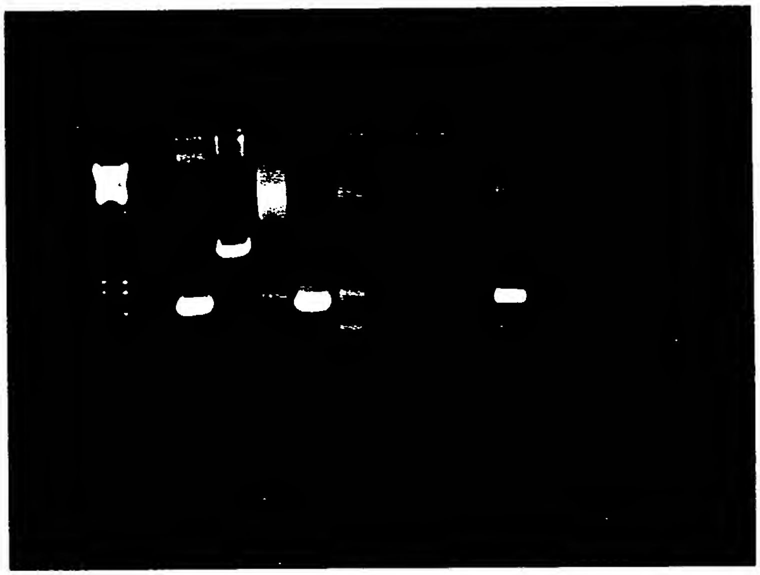
EXHIBIT

D61

03-12-97

Mint preps of small amount (plate) of 293/TTF infected with slow growing viruses p82(?).

PCR, primers 10, 11.



1) ladder.
2) blank (H2O)
3) pF6140.
4) p82
5) mock.
6) wt.
7) 8212
8) 8214
9) 8215
10) 82-16
11) 82-17.

Conclusions:

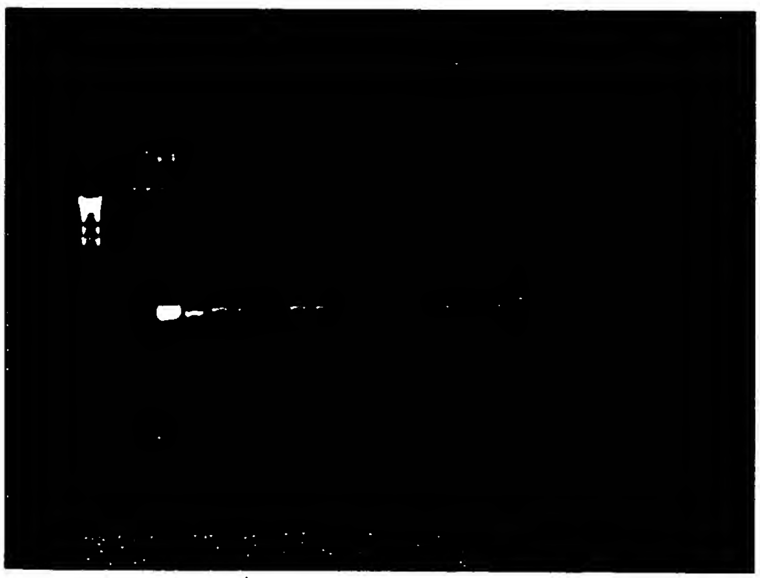
blank - clean.

in 82-12, 82-17 - band, corresponding to wt. and weak additional band corresp. to rec. (does not present in wt!!! something of a bit smaller MW present in mock

EXHIBIT D62

3-17-97

PCR analysis of p82 resulting plaques & primers (UD) 8, (UD) 9 — SPB-promoter-specific).



1) ladder
2) blank
3) pF6140
4) p82
5) mock
6) wt 1101/1107
7) 821
8) 822
9) 823
10) 824
11) 825
12) 826
13) 827
14) 828
15) 829
16) 8210
17) 8211
18) 8212
19) 8214
20) 8215
21) 8216
22) 8217

EXHIBIT D63

Conclusion: there is SP-B in 293 cells, viruses are rather wt than recombinants, need to use (UD) 9, (UD) 11 combination of primers (9 – SP-B specific, 11 – Ad specific)

3-18-97

Sequencing

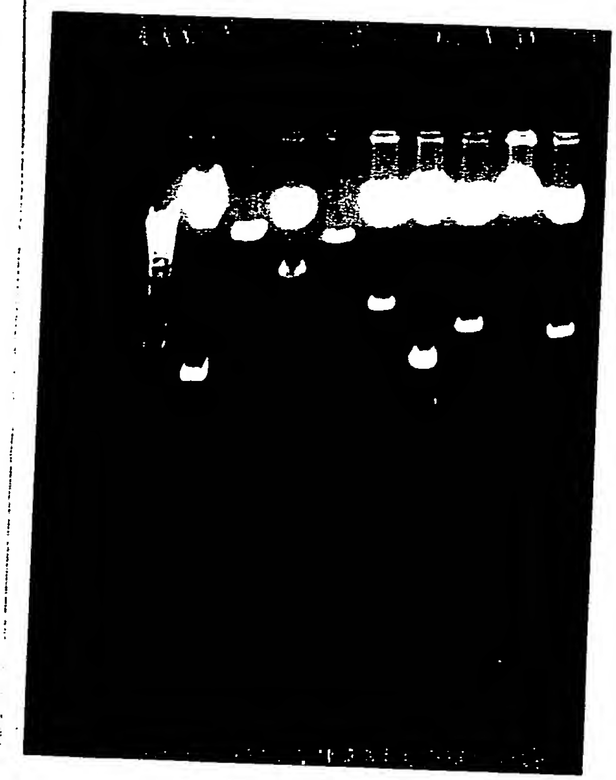
KD 9 (p21, primer KD10)

KD 10 (p21, primer KD11)

KD 11 (p111, primer KD4)

KD 12 (p111, primer KD5)

Restriction pattern of CsCl preps. of p101, p111.



1) 1kb Ladder
2) L2/ RI + NdeI
3) p31/ RI + NdeI
4) p101 / RI + NdeI
5) p42 / RI + NdeI
6) p111 / RI + NdeI
7) L2 / PacI + SpeI
8) p101 / PacI + SpeI
9) L2 / SpeI + XbaI
10) p111 / SpeI + XbaI

3-21-97

Analysis of sequences KD 9, 10, 11, 12.

Everything is fine with p111. As predicted.

p21 – sequence with KD11 primer is fine.
– sequence with KD 10 primer shown that Tn5 E. coli sequence is going after Ad nt 35858 and up to the end of sequence (~500 bp).

⇒ see next page

⇒ $\boxed{\text{p626 and p82 are not responsible for virus}}$

Need to check pBK611 for the same mutation.

EXHIBIT

tabbies

D65

3/27/97

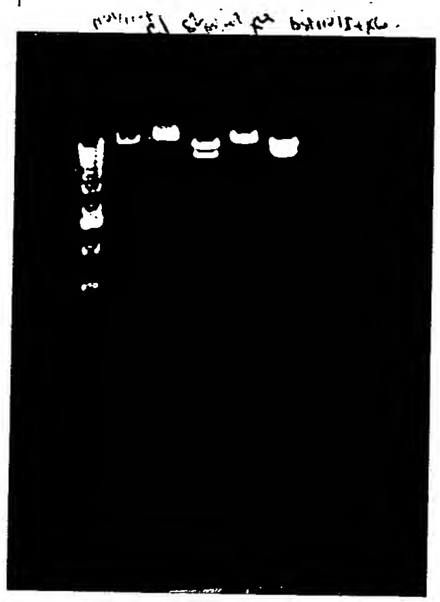
3-20-97 Plaques. (experiments of 02/05 02/02)

626 M 1-12 (12)

626 c 1-6 (6)

11 M 1-4 (4)

3-25-97



1) Ladder 1kb

buffer - ML

2) 1101/1107 DNA / EcoRI + SpeI

3) p82

4) p82 / Bst 1107 I

5) p82 / XbaI

6) p82 / Bst 1107 I + XbaI.

There is Bst 1107 I site in p82 at Ad 29012 !

2nd is after SPB promoter!

3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI
8X 6cm dishes.

3 dishes 101 (for 1 dish) ~~200 µl~~ 250 µl HEBS 2X + 200 µl H_2O + 10 µl 101

3 dishes 111 (for 1 dish) 200 µl HEBS 2X + 200 µl H_2O + 10 µl 111

2 dishes control (for 1 dish) 250 µl HEBS 2X + 200 µl H_2O ——

+ 10 µl 1101/1107 + 25 µl $CaCl_2$.

with Wil

4/7/97

Plaque essay of 544 on 253.

day 16

309 - (~2 / ~1) - plaques are confluent (too many plaques per dish) (CsCl stock)

1101/1107 (~2 / ~4) - Same as with 309 (CsCl and crude lysate)

544 (titer ~2 / ~1 is about 1-2 orders of magnitude lower than one in wt / 309, 1101; plaques appeared ~1 day later than in wt (~day 5) and are a bit smaller.

(day 16 size ~ 8mm)

7001 (CsCl) (titer is 1-2 orders of m. lower than predicted for wt) ~2/~4

plaques are tiny (day 16 ~ 1-2 mm diameter)

4/8/97

Ligation

12ex 1) 82 (10μl) + PCR product ITR (10μl)
Bst (part) + Xba from 140 (4) (6,17)

2) 87 (5μl) + ——"——

4/16/97

Ligation 12 ex: vector 87/XBa + Bst (partial) + CIP

82 / Xba + Bst (part) + CIP upper fragment.

82 / XBa + Bst + CIP lower fragment

EXHIBIT D67

4/17/97 Sequencing.

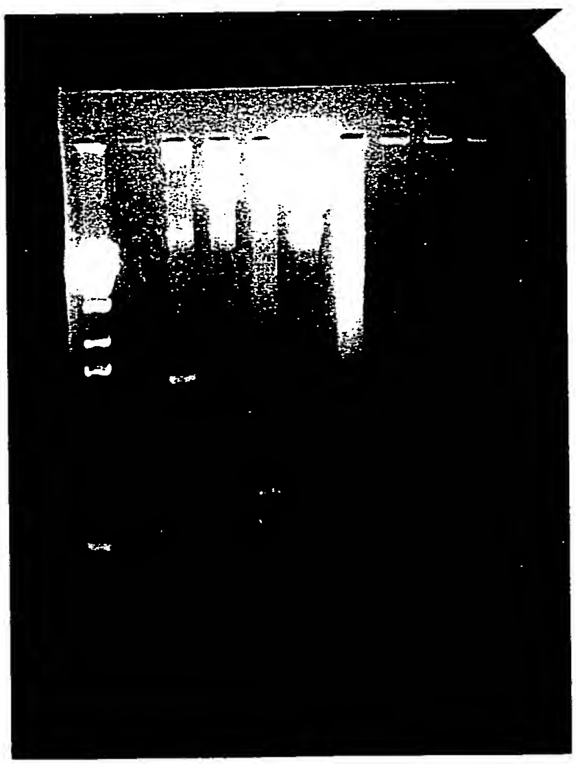
KD (14) – (pB4611, primer KD 10)

KD (15) – (pB4610, primer KD 10)

KD (16) – Nippen's Gal4 large plasmid, primer KD 16

KD (17) – p82, primer KD 10

KD (18) – p111, primer KD 10.



Mini preps of 101-1 and 111-1,

PCR, primers 1,5.

1) ladder.

2) blank

3) pB4610.

4) p101

5) 101-1

6) p111

7) 111-1

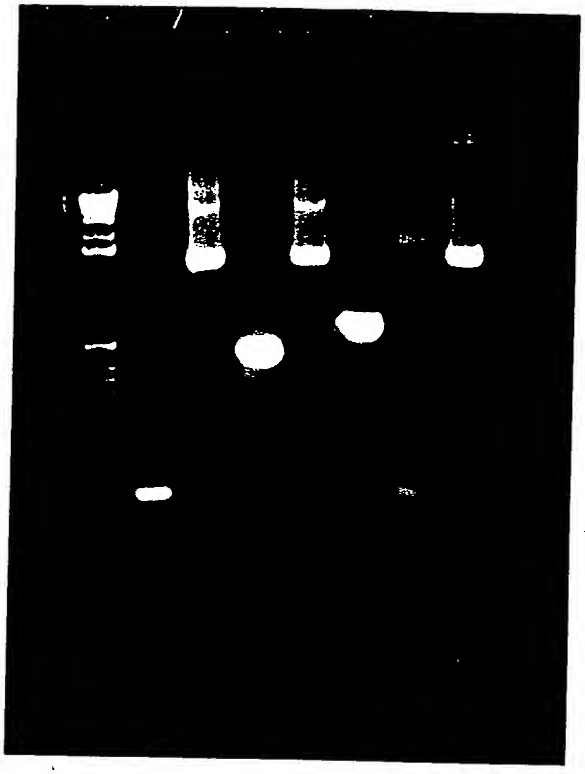
p101, p111 (111-1) – PCR didn't work

for 101-1 product is as expected for 111

Mixed tubes 101-1, 111-1 ⇒ ? or what?

EXHIBIT

D68



PCR primers 1, 7.

1) Ladder
2) blank.
3) 1wo.
4) 54
5) p101
6) p111.
7) 101-1
8) 111-1.

in 111-1 band is as in wt (or 101)
in 101-1 band is higher than wt.

4/21/97

PCR, primers 1, 7.

1) Ladder.
2) blank
3) p1wo.
4) p54
5) p101
6) p111
7) 101-1
8) 111-1.

Conclusion:
this time everything is as expected.

9) 111-2
10) 111-3
11) 111-4
12) 101-1

} plaques.

4/22

Sequencing:

KD 19 - Chip's pED vector, primer CFC II 6

KD 20 - pBHG10, primer KD10.

KD 21 - p82, primer KD 16

KD 22 - p82, primer KD 17

KD 23 - p21, primer KD 16

KD 24 - p21, primer KD 17

Results of previous sequencing:

KD 14 (pBHG11, primer KD 10) - sequence terminates on same point where TAS sequences start in p21.

KD 15 (pBHG10, primer KD 10) - same as ↑

KD 16 (Mippers large GAL4 plasmid, primer KD 16) - same as ↑ but there is some sequence which is what?

KD 17 (p82, primer KD 10) - sequence of 30-B promoter, need to repeat with KD 16 primer.

KD 18 (p111, primer KD 10) - sequence is perfect as predicted!

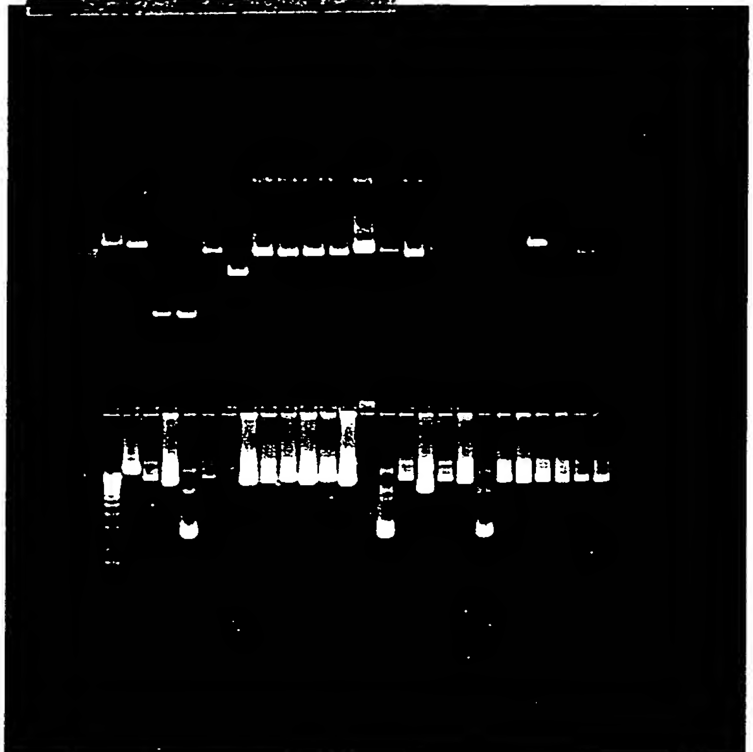
Sequences 19-24 - early termination everywhere
⇒ new protocol doesn't work.

EXHIBIT
D70

4/22
minipreps of 12 ex.

1) ladder,
2) p82
3) 87-1
4) 87-2
5) 87-3
6) 824ex1 (1-9)
7) 824ex2 (1-12)
8)
9)

1) ladder
2) p82.
3) 82L ex1 (1-12)
4) 82 Lex2 (1-12)

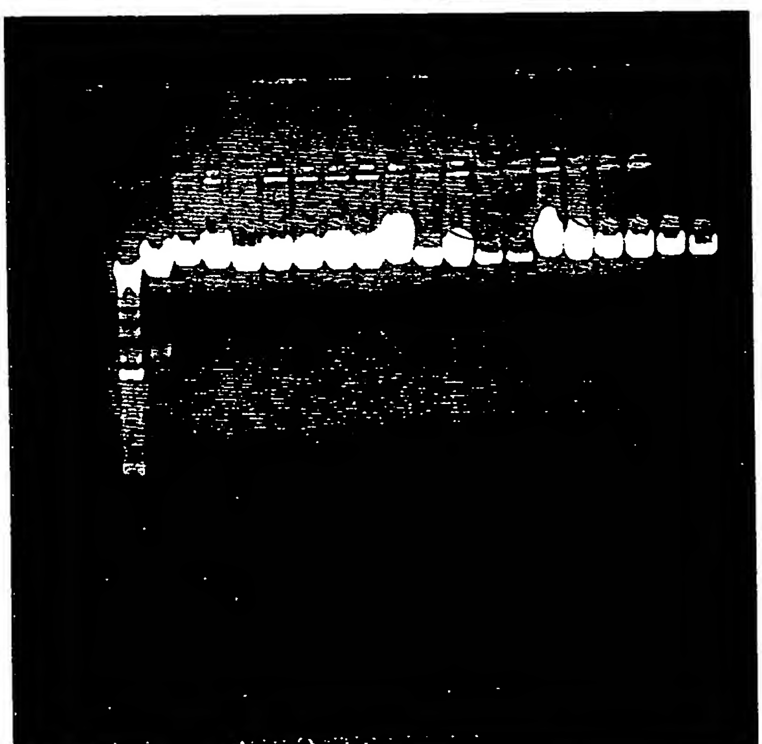


4/23 Restriction analysis of clones 12 ex

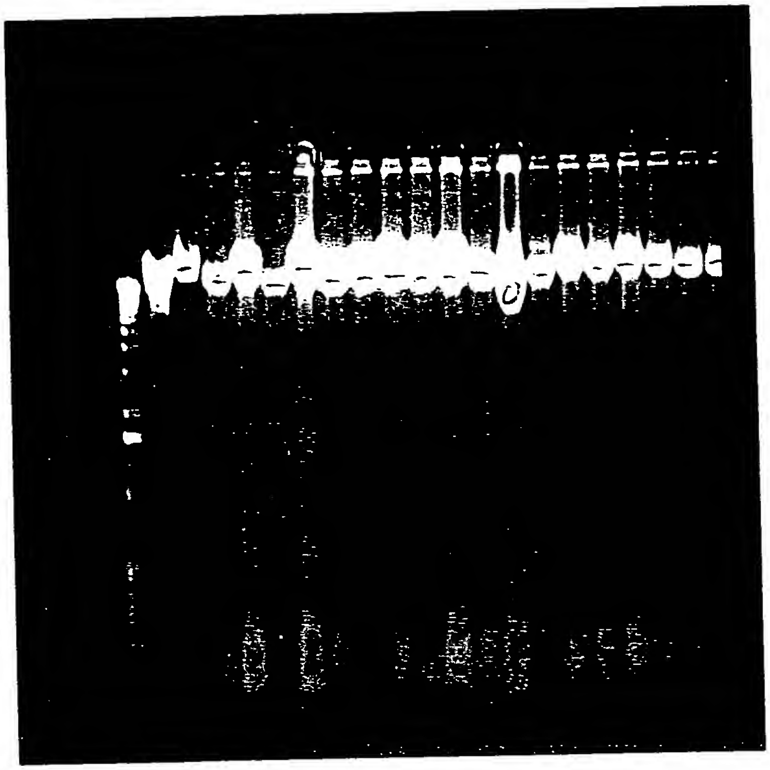
1) ladder
2) p82, R1
3) p82, Xba
4) 871
5) 8241-1
6) 8241-3
7) 8241-4
8) 8241-5
9) 41-6
10) 41-7
11) 41-8
12) 41-9
13) 42-1
14) 42-4
15) 42-5
16) 42-7
17) 42-8
18) 42-9
19) 42-10
20) 42-12

EcoR1

EXHIBIT
D71



Conclusions; 82/R1 - partial digestion.
clones - no small bands. ?
? possibly - recombinants.
Marked bands have lower MW than p82/Xba)



1) Ladder.
2) p82/RI
3) p82/XbaI
4) L1-1
5) L1-2
6) L1-4
7) L1-6
8) L1-7
9) L1-8
10) L1-9
11) L1-10
12) L1-11
13) L2-2
14) L2-3
15) L2-4
16) L2-5
17) L2-7
18) L2-8
19) L2-9
20) L2-10
21) L2-11
22) L2-12

EcoRI

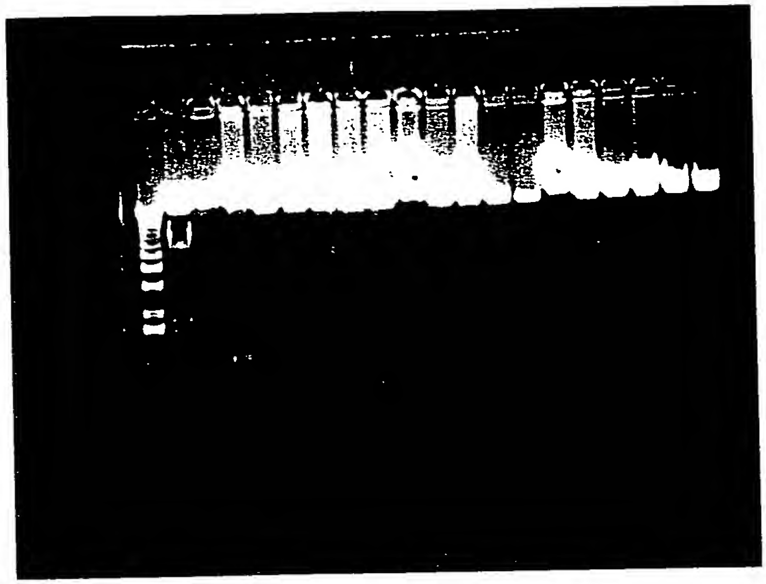
Conclusion: p82/RI - partial digestion.
clones - no small bands.

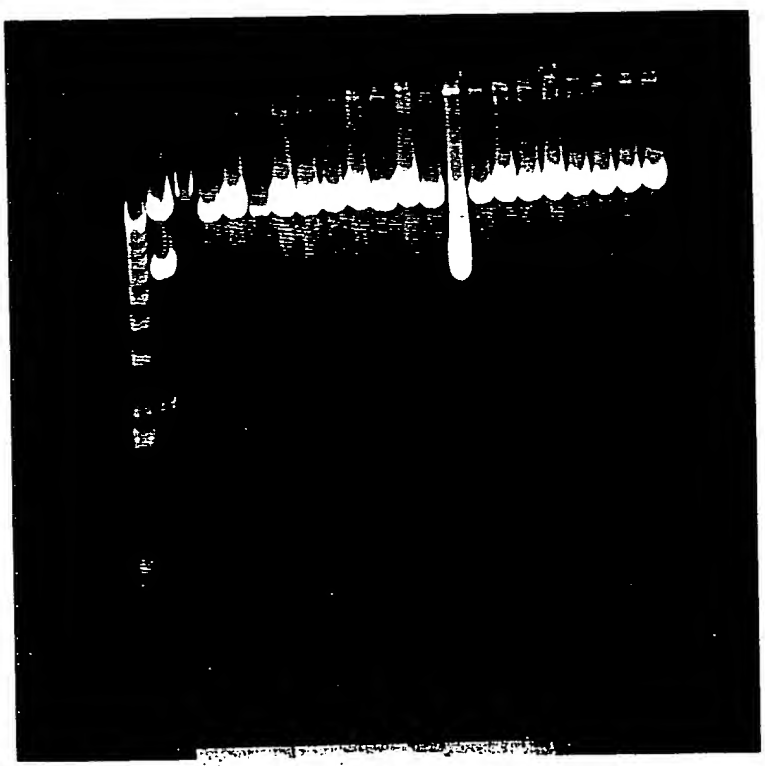
L2-3 - exclude from ~~further~~ further analysis.

Try NdeI - XbaI ~~hydro~~ digestion.
expect 5 kb fragment in rec,
6 kb fragment in 82 (wt)

EXHIBIT
D72

4/29





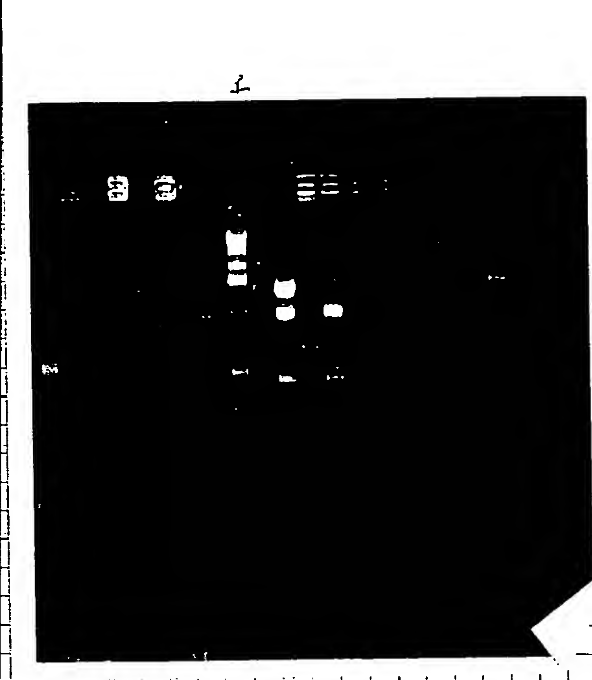
Same as previous 2 pictures,
1) ladder
2) p82 / Xba + Nde
3) p82 / Nde.
4) clones / Xba + Nde
↓

Conclusion ⇒ all these clones are junk, start cloning of ITR into 223 where BstII is unique, then put into resulting plasmid Bam-Nde fragment from pRG140 (unique site again).

Probably, most of them are dl Bst - Bst clones?
But where is ~~vector~~ ~~Background~~?

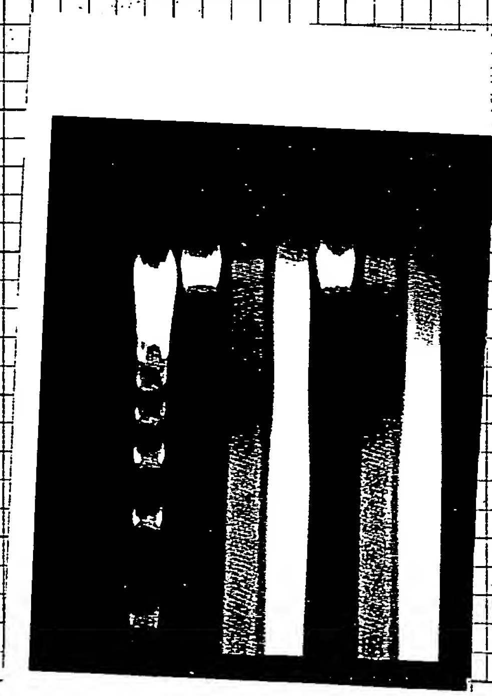
EXHIBIT
D23

KD2 is made
4/24/97



4/24
Restriction digestion
of pcr products
with Pac I
(pcr, primers 1, 7)

1) ladder
2) pREGlw
3) p101
4) p111
5) 101-1

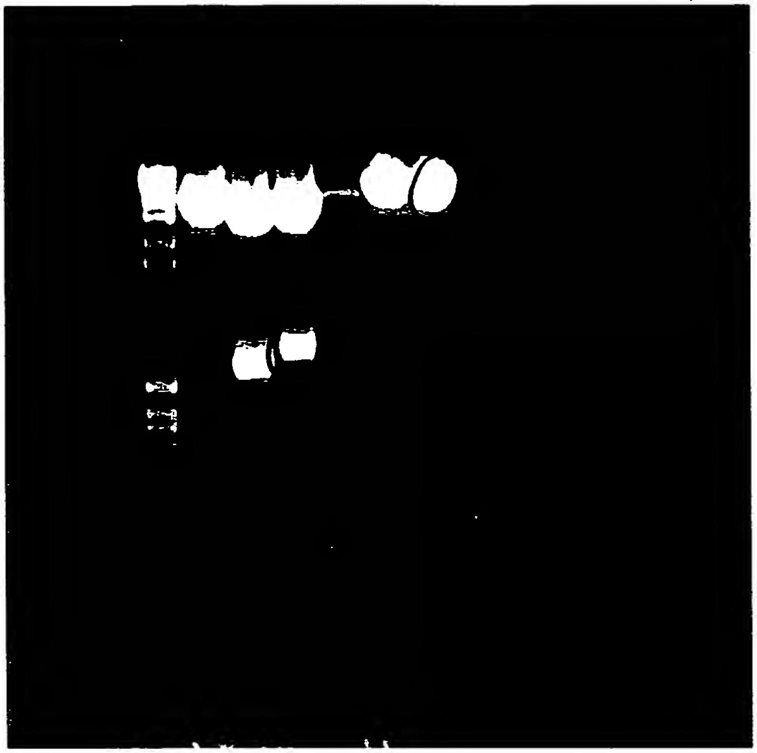


1) ladder
2) 1101/1107 } Pac I + BamHI
3) 101-1
4) 111-1

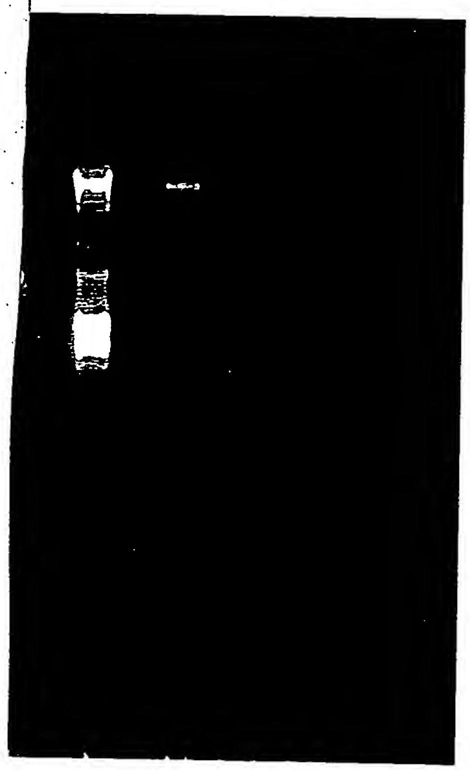
5) 1101/1107 } BamHI + XbaI
6) 101-1
7) 111-1

Conclusion: 101-1 is rec.
111-1 ⇒ ?

4/26.



1) ladder
2) pGRII/gp19kAds (Terry) / Kpn I
3) ________ RI
4) ________ Bam + Xba
5) pCI/gp19k (Terry) / EcoRI
6) pcDNA3 / EcoRI
7) pcDNA3 / Bam + Xba.

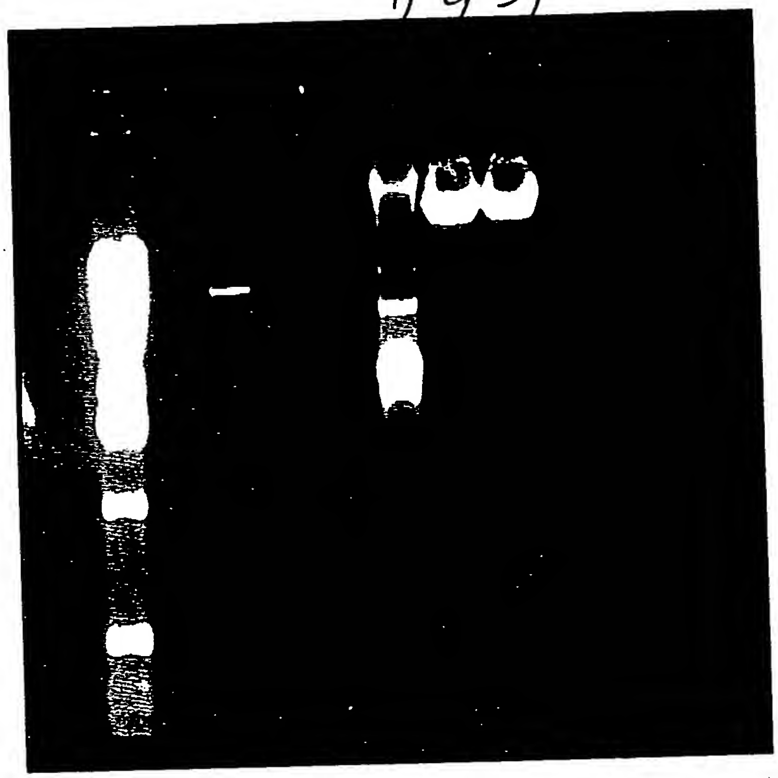


1) ladder
2) gp19k Ads Bam-Xba fr.
3) pcDNA3 Bam-Xba vector.

Ligation:

1) vector 5µl
2) vector lig 5µl
3) vector + insert
   5µl 5µl

1) 2) 3)

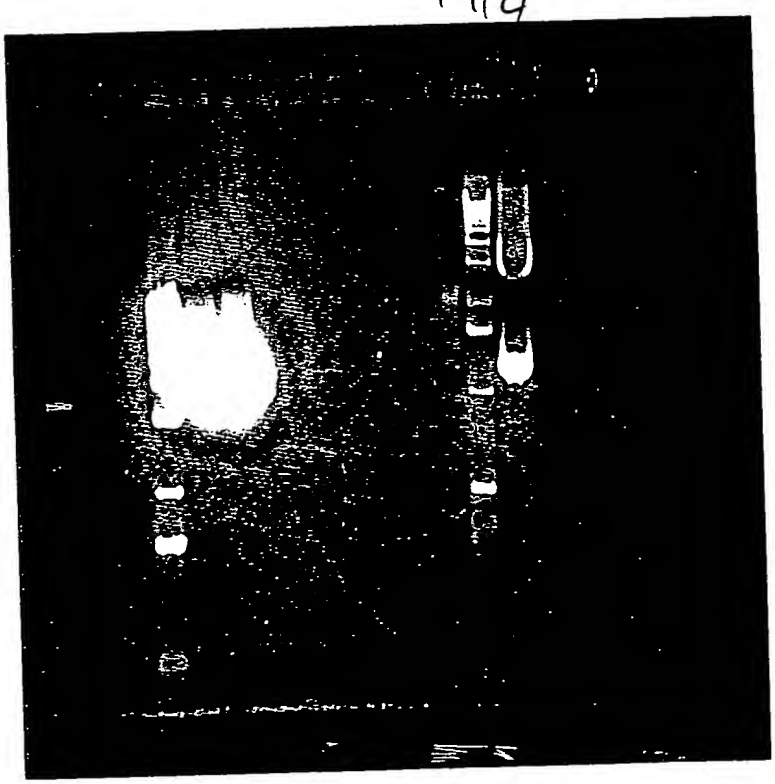


1) Ladder
2) pCRII/gp19a Ad5 / Bam + BstI107I
3) — " — / BstI107I + Xba

It doesn't look like Ad5 gp19a.

check with PstI
check with Terry!

1) 2)



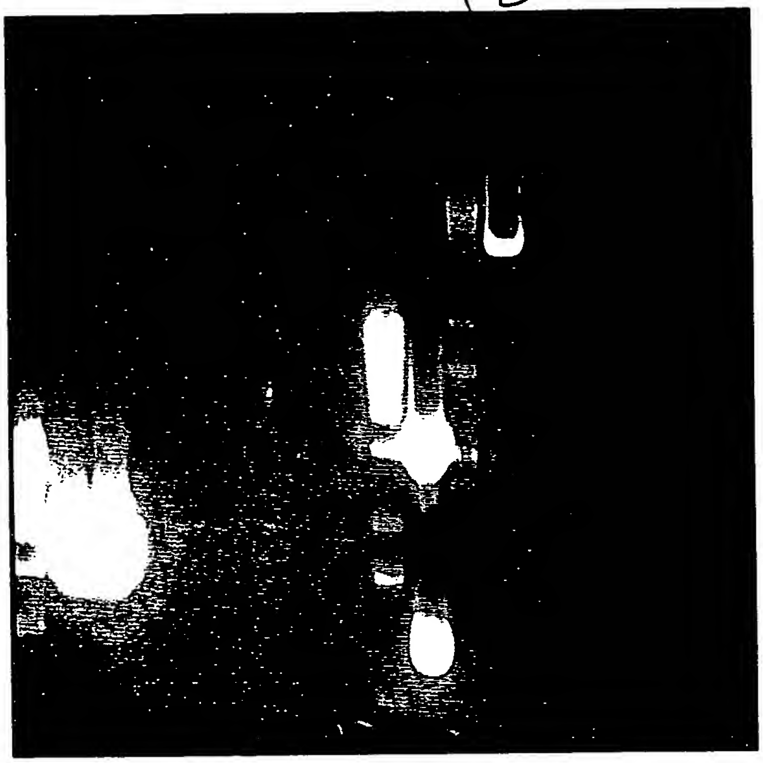
4/27

1) Ladder
2) pCRII/gp19a Ad5 / Pst

there is a small fragment!

this is Ad2 gp is a in reverse orientation!?

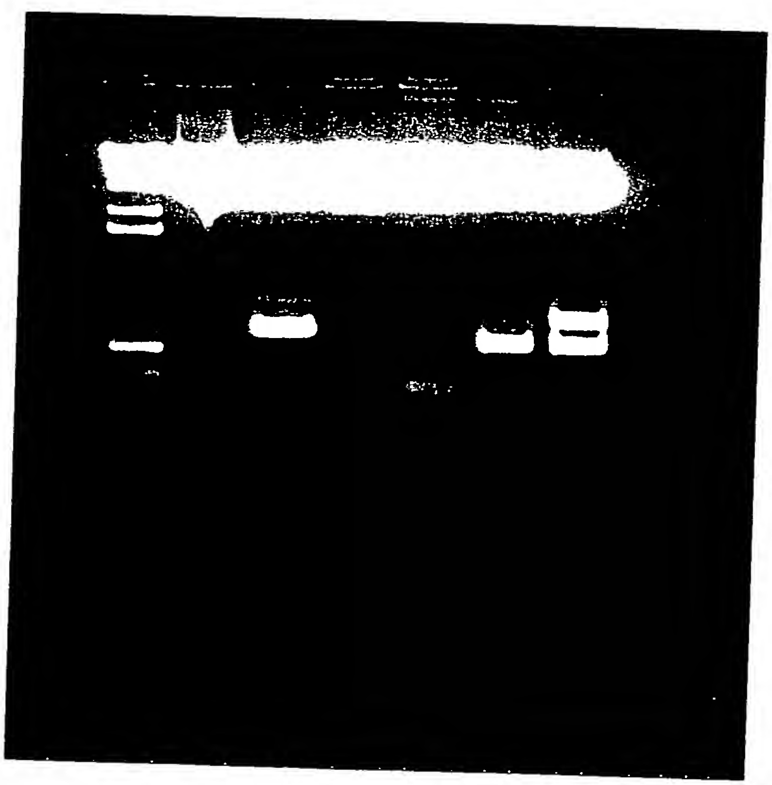
check with HindIII



4/28.

1) ladder.
1) pCR4/gp19k/HindIII

~500 bp fragment?



4/28.
PCI/gp19k
restriction digestion

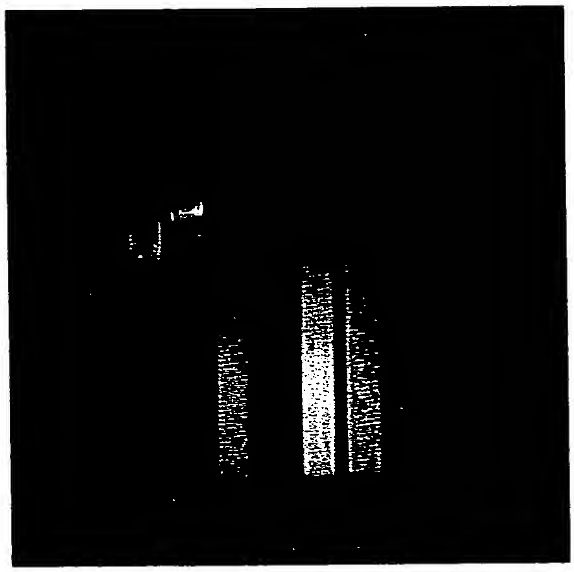
1) ladder
2) pcI/gp19k
3) ——"—— / EcoRI
4) ——"—— / KpnI
5) ——"—— / PstI
6) ——"—— / HindIII
7) ——"—— / HindIII BamHI

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

EXHIBIT
D77

KO 3 is made
4/30/97

4/29/97 Restriction digestion of 111 plaques

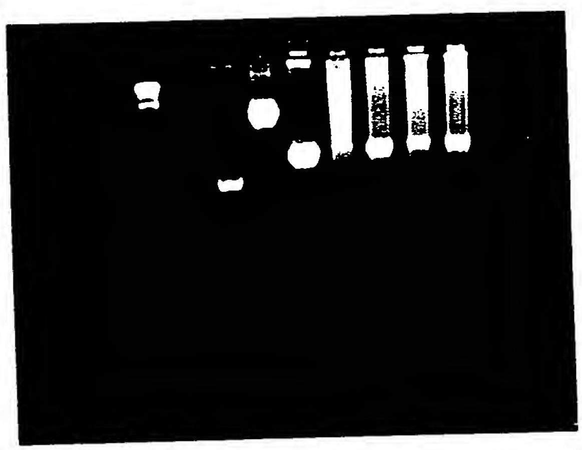


1) ladder
2) 1101/1107 DNA
3) 111-1 (new prep)
4) 111-2
5) 111-3
6) 111-4

Bam H1 + Xba I

111 - 2, 3, 4 - look like recombinants

4/30/97
PCR analysis of 111 plaques,
Primers KO 1, KO 7



1) ladder
2) blank
3) p54
4) pFG/ba
5) p111
6) 111-1
7) 111-2
8) 111-3
9) 111-4

~~Conclusion~~ is => all plaques are

EXHIBIT
tabbies
D78

4/30. Ligation:

cloning experiment 14. (14 ex).

1) Vector p223 / Bst 1107 I + XGa I (PEG prep.)

2) ~~Vector~~ p223 / Bst 1107 I + XGa I (WKit prep).

3) Insert 1101/1107 PCR ITR / Bst 1107 I + XGa I
(without gel purification).

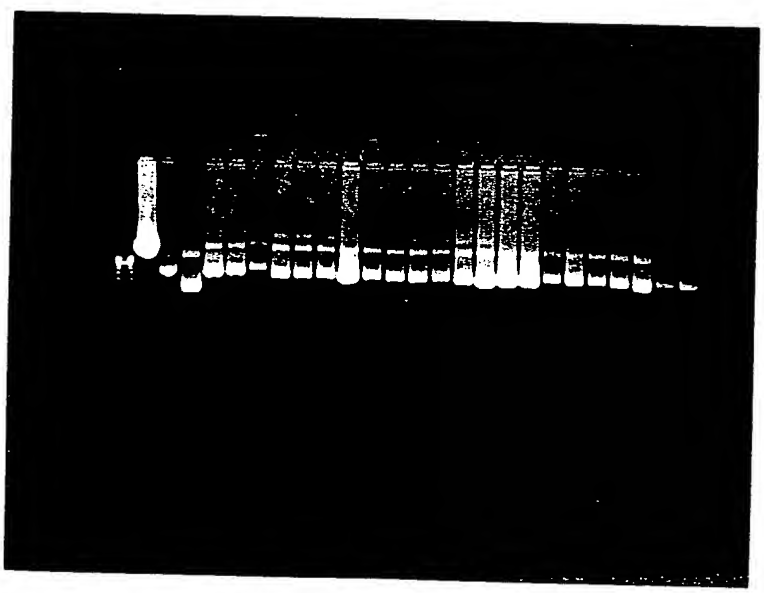
5/5. Comparing growth properties of: (plaqueing efficiency)

54 (4) Controls 309
101 (1) 1101/1107
111 (1) 7001
111 (2) m1 m41 (pm 734.1)

5/6/97

Minipreps of 14ex.

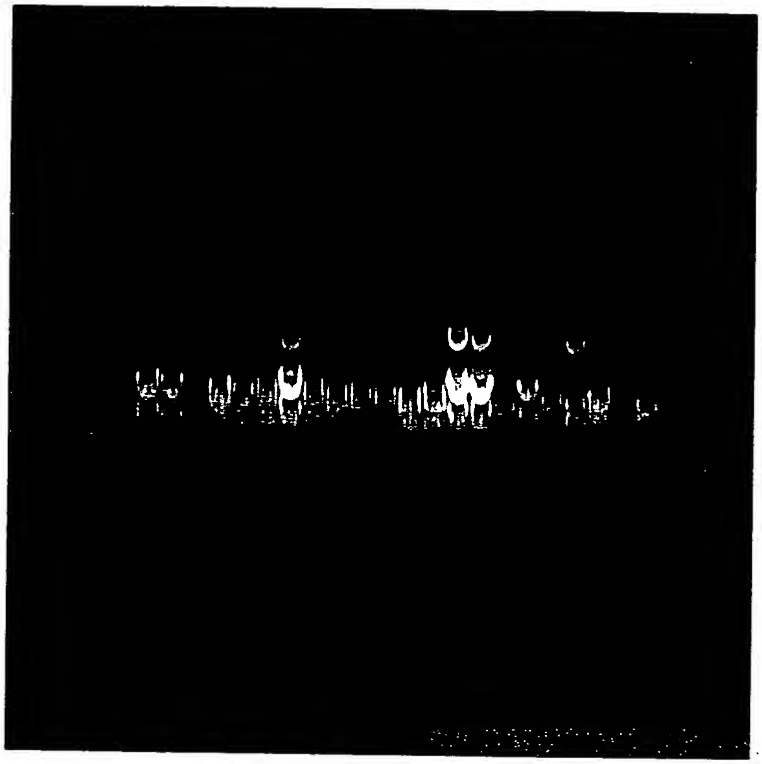
1) ladder
2) p82
3) p223
4) 12ex-1
5)
6) 14ex 1 (1-3)
7)
9) 14ex 2 (1-19)
10)
↓
26)



→ 12ex-1 - junk.

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

EXHIBIT
D79



4/6/97

1) ladder

↓ Xba + Nde restriction of ~~2 ex~~, 14 ex clones

2) p223

141 (1-3)

142 (1-20)

Xba + Nde.

Conclusion:

141-3

142 -

1, 2, 3,

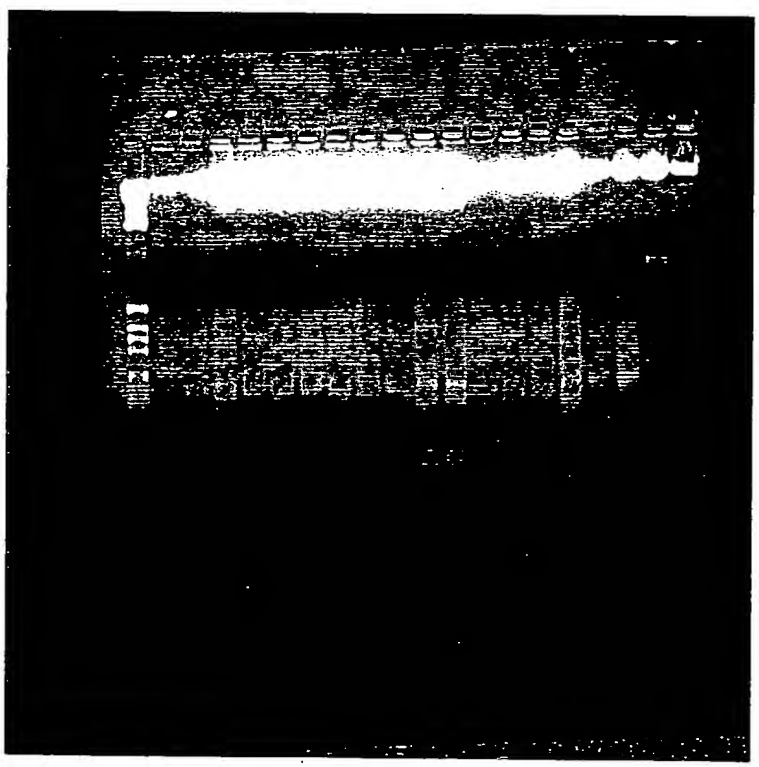
5, 6, 7, 8, 9, 10,

13,

15, 16, 17, 18, 19 -

EXHIBIT D80

Comments



4/7/97

1) ladder
2) p223
3) 141(3)
4) 142(1)
5) 142(2)
6) 142(3)
7) 142(5)
8) 142(6)
9) 142 (7)
10) 142 (8)
11) 142 (9)
12) 142 (10)
13) 142 (13)
14) 142 (15)
15) 142 (16)
16) 142 (17)
17) 142 (18)
18) 142 (19)
19) 141 (1) (wt)
20) 142(20 wt)

All rec clones from ↑ are rec-ts.

Bst 217I (Bst1107I) + Xba I

418

Sequencing

kd 25 - p141(3) - primer kd 16
kd 26 - p141(3) — " — kd 17
kd 27 - p142(1) — " — kd 16
kd 28 - p142(1) — " — kd 17

sequencing Didn't work

sequence is OK.

4/14. preparing vectors pBSSK(+)/Sma I MC! 30°C
p142(1) Bam + Nde. MC

rec 700, pm 734.1 predicted fragments lenght for Srf I - Nde I. digestion:

19549
7981
4846
3559

Ligation:

Cloning exp. 15 (p142(1) Bam + Nde + pFG140 Bam-Nde B fr)

Cloning exp 16 (pBSSK Sma + rec700/Srf + Nde + T4 pol)

Cloning exp 17 (pBSSK Sma + pm 734.1/Srf + Nde + T4 pol

5/16. Results of transform. DH5α with 15, 16, 17 ex:

15 V - 1
15 VL - 14
15 ex - 25

15-2 - MW as 82
others either wt 142-1 or small plasmids

16 V - 0
16 VL - 2R + 1W
16 ex - 5R + 19W
17 ex - 7W

all WT or same MW

4/20

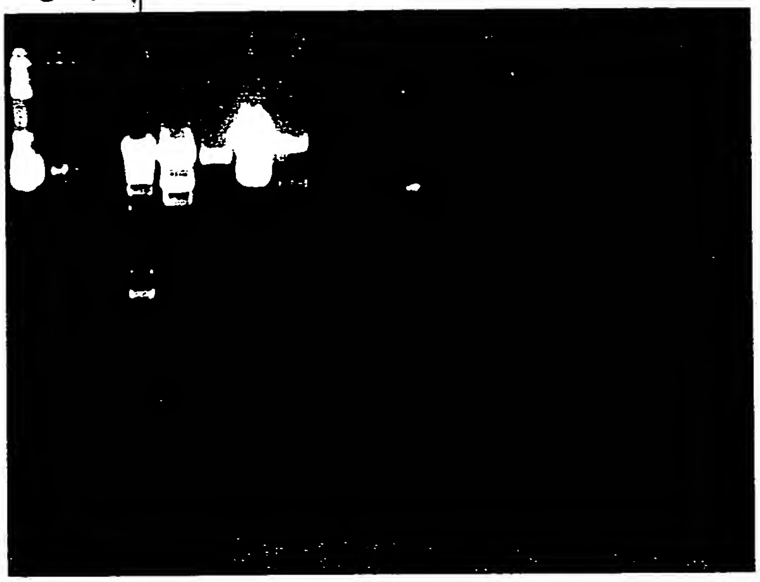
1) p82
2) p152

[illegible]

1) ladder
2) p82 } BamH +
3) p152 } Nde I
4) p82 } Nde I +
5) p152 } Xba I

Conclusion: 152 is ok, making cell prep.

1 2 3 4



5/23 Minipreps of 17ex

17ex -2 (Klenow - excise fragment from gel)

1) ladder
2) pBSK(+)
3) 17ex
↓
1-12
17ex2
1-12

take 172-1, 10, 11, 12

172-5 ?
172-8 ? } possibly seen junk.

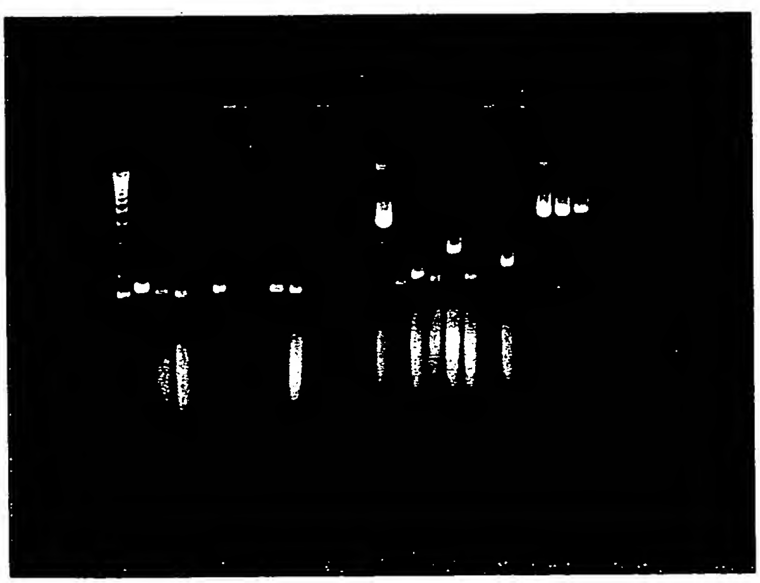
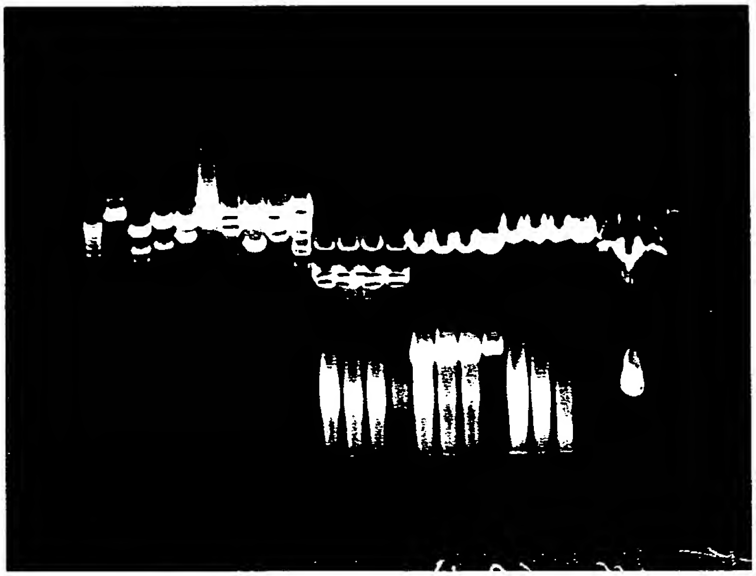


EXHIBIT
D82

5/27/97



1) ladder
2) p82
3) p82 / Bam + Nde
4) p82 / Nde + Xba
5) p82 / bst 1107I
6) p152
7) p152 / Bam + Nde
8) p152 / Nde + Xba
9) p152 / Bst 1107I
} maxi prep (SCL)
10) 734.1 / Srf + Nde
11) 1721
12) 17210
13) 17211
14) 17212
} Sal + Spe
15) 1721
16) 17210
17) 17211
18) 17212
} RI
19) 1721
20) 17210
21) 17211
22) 17212
} Xba
23) pcigp191c / Xba + Xho
24) 1101/1107 / Spe + EcoRI.

Conclusion: 152 is as expected.
17ex clones have correct insert in same (direct) orientation (EcRI digestion) Xba dosn't cut insert due to methylation of Xba site.

the insert could be cut out with BamI - XbaI to clone it into pCDNA3.1 Zeo(+) BamI - XhoI sites.

EXHIBIT
D83

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

5/28/97

Transfection 1101/1107 EcoRI + SpeI + p152

6 dishes 293/TTF
8 dishes 293

293 TTF

2 dishes - control (1 ml)
4 dishes - experiment 2 - TTF (1 ml)
2 - + CMV/TTF (1 ml)

5/29/97

Cloning 18 ex;

172 Bam + Sal I + XbaI (last) ⇒ 3.5 kb (A)
+ CIP
fragment (1 cut) ↘ (buffer D))

pcDNA3.1 BamHI + XhoI ⇒ vector
Zeo
5.0 kb. ↘ (buffer D))

V (5 μl) - 8, VL ~30, ex ~ 300, LL (10 μl) - 0

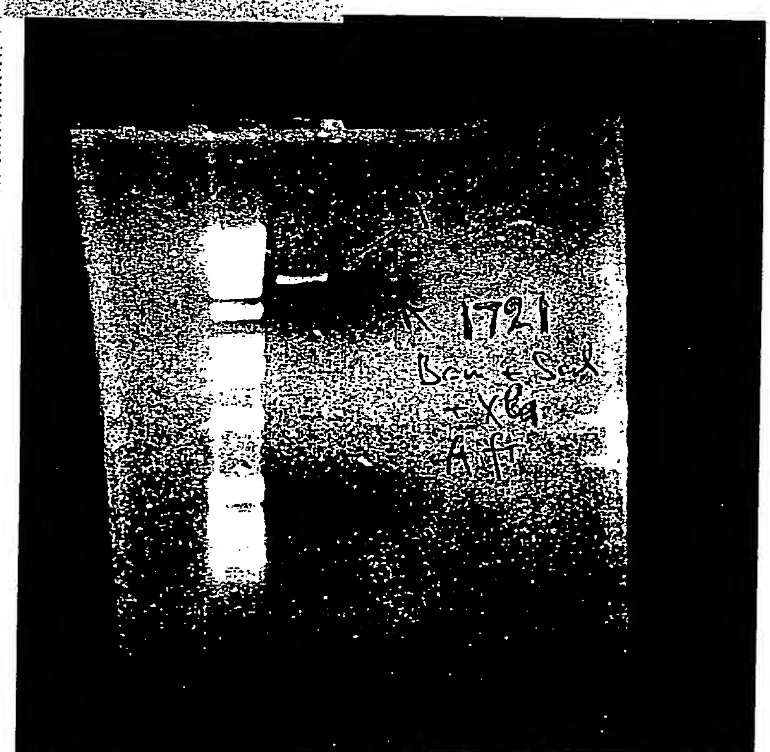


EXHIBIT
D84

First cell culture experiment with KO1,-2,-3 5/15/97

5/8/97 Plaque assay of E3 viruses 293 cells ADD

Day — ~~Number of plaques~~/plaques size (mm)

Virus (~~titer approximate~~) (orders of magnitude)		3	4	5	6	7	8	10	19
309	(~2)	1/1	8/12	~100/1-5	~300/1-5	~500/1-5	Same	compl CPE ~1000	Compl CPE
	(~1)	0/0	0/0	~20/1-2	~50/1-5	~100/1-5	Same	~100	~200
1101/1107	(~2)	0/0	0/0	~50/1-2	~30/1-5	~50/1-5	Same	~100	~900
	(~1)	0/0	0/0	5/1	19/1-2	10/1-2	same	~30	50
7001	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
pm T34.1	(~4)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	~30
	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
544	(~4)	0/0	0/0	10/1	18/1-2	20 (tiny 1-2)	30 (tiny)	~60 (tiny)	~100
	(~3)	0/0	0/0	0/0	1/1	4 (tiny)	Same	9 (~1)	30
101-1	(~4)	6/1	15/1-2	>100/1-5	>300/1-5	>1000	Same	complete CPE	Compl CPE
	(~3)	0/0	0/0	~50/1-5	~80/1-5	>100	Same	>300	>300
	(~2)	0/0	1/1	~15/1-5	~30/1-5	>30	same	>70 large	100
111-1	(~4)	6/2	9/2-8	>100/1-10	>300 (?)	>1000	Same	complete CPE ~10^4	compl CPE
	(~3)	0/0	1/8	~50/1-10	~~80~~ (1-5)	~80	Same	~300	>300
	(~2)	0/0	0/0	1 of 1 (most 1)	30/1-3	~30	same	~50 large	>50
111-2	(~4)	0/0	8/8-5	20/1-2	50/1-5	50	same	~100	>100
	(~3)	0/0	1/1	10/1	20/1-5	30	same	~50	>50
	(~2)	0/0	0/0	0/0	7/1	12	same	23	28

~~Up to day 6 size measurements approximate~~

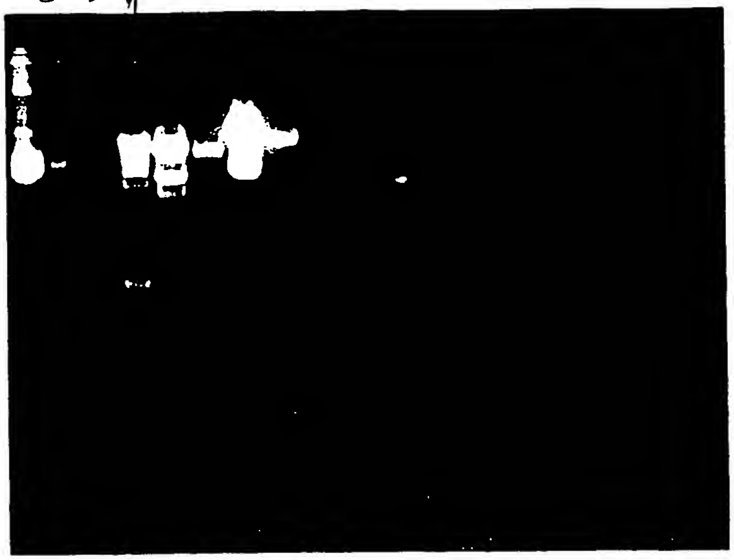
		15	16	18 Fri	19 Sat	22 Tues	23	24	25
309	(~2)	~~CCPE~~							
	(~1)	CCPE							
1101/1107	(~2)	CCPE							
	(~1)	50	60						
7001	(~3)	2	3	3	3	3			
	(~2)	0	1	3	3	5			
pm T34.1	(~4)	~50	~60	~60	~70	~70			
	(~3)	4	7	9	10	16	23	30	
	(~2)	6	6	1	1	2			
594	(~4)	~100	~100	~100	~100				
	(~3)	33	33	33	33				
101-1	(~4)	CCPE							
	(~3)	CCPE							
	(~2)	CCPE							
111-1	(~4)	CCPE							
	(~3)	CCPE							
	(~2)	50	50						
111-2	(~4)	>100	~100						
	(~3)	>50	~50						

EXHIBIT D85

5/15 rec ADP in EB plaque development on A549.

	day = 5	6	7	8 friday	9 Saturday	12 Tues.	12+1	14	15	18
309 (~1)	12	52	~100	~100	~100	CCPE		CCPE		
(~0.1-1)	1	5	14	19	20					
(~0.1-2)	3	7	19	26	27					
101/107? (~1)	0	0	3 small	8	15					
(~0.1-1)	0	0	1	3	3	5	6			
(~0.1-2)	0	0	0	0	1	2	2			
7001 (~3)	0	0	0	0	0	0	0		2	2
(~2)	0	0	0	0	0	0	0			
734.1 (~3)	0	0	0	0	0	8	8	11	15	
(~2)	0	0	0	0	0	4	5	6	9	10
101-1 (~3)	1	4	48	60	~60					
101-1 (~2)	3?	7	20	30	54					
101-1 (~1)	0	2	3	4	7	9		11		
111-1 (~3)	0	6	35	50	~50					
(~2)	0	0	5	9	16					
(~1)	0	0	2	4	4			8	11	
111-2 (~4)	3?	30	70	100	~100					
(~3)	2?	4	16	20	30					
(~2)	0	0	1	4	4	6			7	
(~1)	0	0	0	0	0	0				

12 3 4



4/20

1) p82
2) p152
~~[illegible]~~ run:

1) ladder
2) p82 } BamHI +
3) p152 } NdeI
4) p82 } NdeI +
5) p152 } XbaI

Conclusion: 152 is ok, making cell prep.

5/23 Minipreps of 17ex

17ex-2 (Klenow
— excise fragment from gel

1) ladder
2) pBSK(+)
3) 17ex
↓
1–12
17ex2
1–12

take 172-1, 10, 11, 12
172-5? } possibly
172-8? } some junk.

[illegible], 3/5/97, 5/21, 97

Day (plaques/)

Virus	5	6	7	8	9	10	11	12	13	14	15	16	17	18
309	4/8.5	12/25	33/70	45/95	47/100									
~~#~~ 1101/1107 7001			16/19	14/59	18/85			20/95	21/100					
734.1								12/50	13/54	17/70	24/100			
544		14/33	25/54	41/97	42/100									
101-1	3/7	9/20	23/53	34/74	41/95		43/100							
111-1	2/7	4/13	16/53	20/66	30/100									

EXHIBIT

D88

5/15/97 Plaque development on 293. (% plaques)

Virus \ day 4 5 6 7 ~~8~~ 10 14 15 16 18 19 22 23 24

309 10 25 50 ~~100~~ 100

1101/1107 8.3 16 50 83 100

7001 ~~25 50 75 100~~ ?

734.1 (~~13.5 23.5 30 33 55 76 100~~) !

544. 3 12 27 90 100

101-1. 1 15. 30 70 100

111-1 ~~20 60 100~~ ?

111-2 21 36 69 84 96 100.

5/15/97 ↑ Same, A549.

5 6 7 8 9 12 13 14.

309 11 25 70 96 100

1101/1107 16 50 83 100.

~~7001~~
734.1 ~~70 76 100~~ ?

101-1 18 27 36 63 82 100

111-1 25 56 100.

111-2 16 66 100

5/8/97 Plaque assay of ES viruses ADD 293 cells

Day - ~~Number~~ of plaques/plaques ~~size~~ size (mm)

Virus (titers approximate) orders of magnitude

Virus		3	4	5	6	7	8	10	14
309	(~2)	1/1	8/12	~100/1-5	~300/1-5	~500/1-5	same	compl CPE ~1000	compl CPE
	(~1)	0/0	0/0	~20/1-2	~50/1-3	~100/1-5	same	~100	~200
1101/1107	(~2)	0/0	0/0	~50/1-2	~50/1-5	~50/1-5	same	~100	~500
	(~1)	0/0	0/0	5/1	10/1-2	10/1-2	same	~30	50
7001	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
pm T34.1	(~4)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	~30
	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
544	(~4)	0/0	0/0	1/1	18/1-2	20 (tiny)	30 (tiny)	~60 (tiny)	~100
	(~3)	0/0	0/0	0/0	1/1	4 (tiny)	same	9 (tiny)	30
101-1	(~4)	6/1	15/1-2	>100/1-5	>300/1-5	>1000	same	complete CPE	compl CPE
	(~3)	0/0	0/0	~50/1-5	~80/1-5	>100	same	>300	>300
	(~2)	0/0	1/1	~15/1-5	~30/1-5	>30	same	>70 large	100
111-1	(~4)	6/2	9/2-8	>100/1-10	>300 (?)	>1000	same	complete CPE ~10^4	compl CPE
	(~3)	0/0	1/8	~50/1-10	~80 (1-5)	~80	same	~300	>300
	(~2)	0/0	0/0	10/1 (most 1)	30/1-3	~50	same	~50 large	>50
111-2	(~4)	0/0	8/8-5	20/1-2	50/1-5	50	same	~100	>100
	(~3)	0/0	1/1	10/1	20/1-5	30	same	~50	>50
	(~2)	0/0	0/0	0/0	7/1	12	same	23	28

~~up today 6 size measurements approximate~~

Virus		15	16	18 Friday	19 Sat	22 Tues	23	20	25
309	(~2)	CCPE							
	(~1)	CCPE							
1101/1107	(~2)	CCPE							
	(~1)	~~25~~ 50	60						
7001	(~3)	2	3	3	3	3			
	(~2)	0	1	3	3	5			
pm T34.1	(~4)	~50	~60	~60	~70	~70			
	(~3)	4	7	9	10	16	23	30	
	(~2)	0	0	1	1	2			
544	(~4)	~100	~100	~100	~100				
	(~3)	33	33	33	33				
101-1	(~4)	CCPE							
	(~3)	CCPE							
	(~2)	CCPE							
111-1	(~4)	CCPE							
	(~3)	CCPE							
	(~2)	~50	50						
111-2	(~4)	>100	~100						
	(~3)	>50	~50						
	(~2)	32	33						

First cell culture experiment with KO1,-2,-3 5/15/97

EXHIBIT

D90

5/15 Tce ADP i. E3 plaque development on A524:

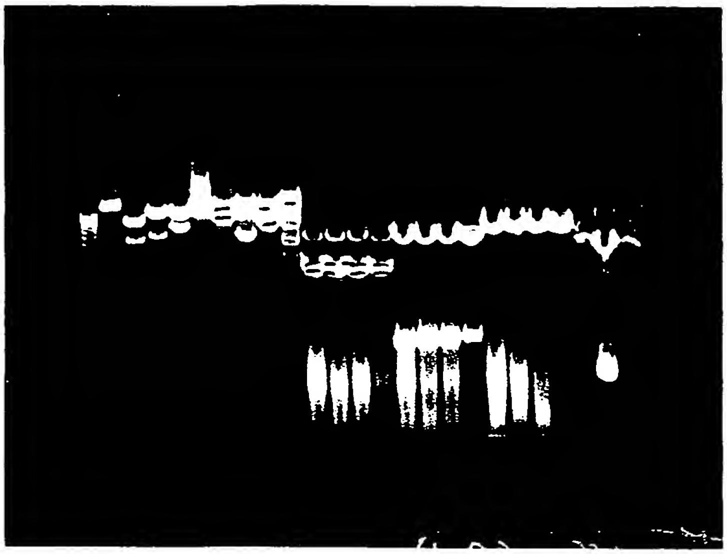
days =	5	6	7	8 friday	9 Saturday	12 Tues.	12+1	14	1[illegible]	[illegible]
309 (~1)	12	52	~100	~100	~100	CCPE		CCPE		
(~0.1+1)	1	5	14	19	20					
(~0.1-2)	3	7	19	26	27					
101/102 (~1)	0	0	3 small	8	15					
(~0.1+1)	0	0	1	3	3	5	6			
(~0.1-2)	0	0	0	0	1	2	2			
7001 (~3)	0	0	0	0	0	0	~~4~~		2	2
(~2)	0	0	0	0	0	0	0			
734.1 (~3)	0	0	0	0	0	8	8	11	15	
(~2)	0	0	0	0	0	4	5	6	9	10
101-1 (~3)	1	11	48	60	~60					
101+1 (~2)	3?	7	20	30	34					
101-1 (~1)	0	2	3	4	7	9		11		
111-1 (~3)	0	6	35	50	~50					
(~2)	0	0	5	9	16					
(~1)	0	0	2	4	4			8	11	
111-2 (~4)	3?	30	70	100	~100					
(~3)	2?	4	16	20	30					
(~2)	0	0	1	4	4	6			7	
(~1)	0	0	0	0	0	0				

EXHIBIT
D91

5/21/97 Plaque assay of E3 viruses on A549;
(monolayer is dying, data are not quantitative)

virus \ Day, plaques		6	7	8	12	14
309	(~2)	~30				
	(~1)	3	12	33		
01/1107	(~3)	0	3	17	27	
	(~2)	0	3	17	30	
	(~1)	0	0	6	7	
001	(~4)	0	0	1	1	6
	(~3)	0	0	0	0	
734	(~4)	0	0	0		
	(~3)	0	0	0	2	3
544	(~4)	14	24	39		
	(~3)	0	4	2	3	3
	(~2)	0	0	0	0	0
1011	(~3)	0	11	28		
	(~2)	0	3	11	12	
111-1	(~3)	0	0	3	5	
	(~2)	0	0	7	14	
111-2	(~3)	0	~~7~~	27		
	(~2)	0	0	1	2	
	(~1)	0	0		0	

5/27/97



1) ladder
2) p82
3) p82 / Bam + Nde
4) p82 / Nde + Xbg
5) p82 / bst 1107I
6) p152
7) p152 / Bam + Nde
8) p152 / Nde + Xbg
9) p152 / bst 1107 I
maxi prep (SCL)
10) 734.1 / Srf + Nde
11) 1721
12) 17210
13) 17211
14) 17212
Sal + Spe
15) 1721
16) 17210
17) 17211
18) 17212
RI
19) 1721
20) 17210
21) 17211
22) 17212
Xbg
23) pcigpl91c / Xba + Xho
24) 1101/1107 / Spe + EcoRI

Conclusion: 152 is as expected.
17ex clones have correct insert in same (direct) orientation (EcRI digestion) Xba dosn't cut insert due to methylation of Xba site.
the insert could be cut out with BamI-XbaI to clone it into pcDNA3.1 Zeo(+) BamI-XhoI sites.

EXHIBIT
D93

5/28/97

Transfection 1101/1107 EcoRI + SpeI + p^{152}.

6 dishes 293/TTF

8 dishes 293

293 TTF

2 dishes - control (1 ml)

4 dishes - experiment 2 - TTF (1 ml)
2 - + cmv/TTF (1 ml).

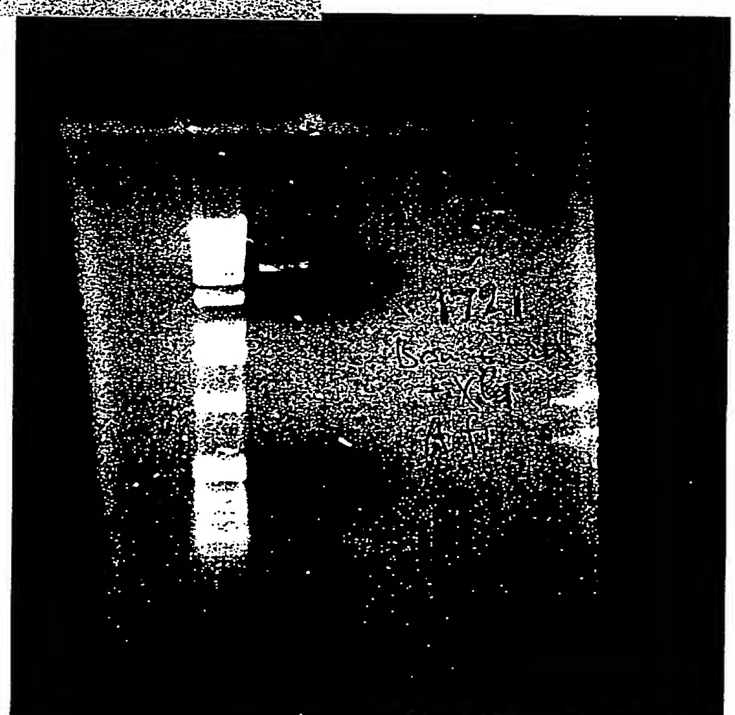
5/29/97

Cloning 18 ex ;

172 Bam + Sal + XcmI (last) ⇒ 3.5 kb (A)
+ CIP
fragment (1 cut). → (buffer))

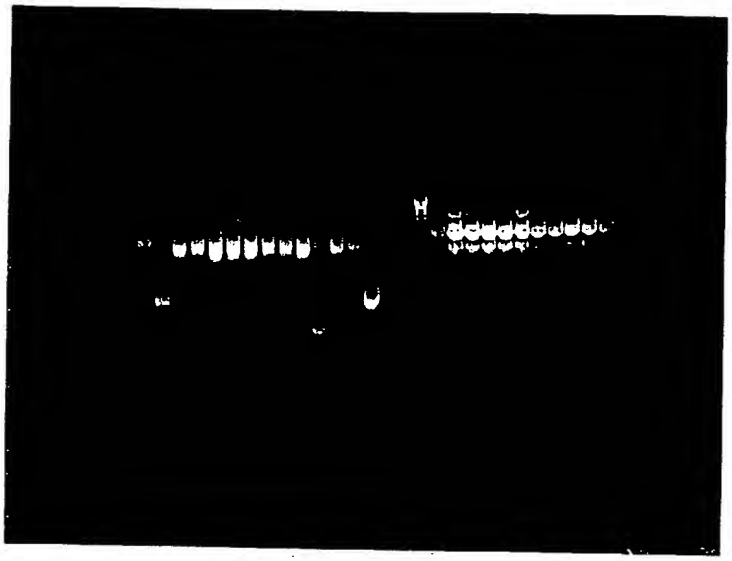
pCDNA3.1 Bam H + XhoI ⇒ vector
Zeo
5.0 kb. → (buffer D))

V (3M) - 8, VL ~ 30, ex ~ 300; LL (10 μl) - 0



6/5/99

18 ex minipreps → 1 - 8, 10, 11 - look like rect's (~~correct~~ MW)



1) ladder
2) ↓ 18 ex (1-12)

Take 1-8, 10, 11.

1) Ladder
2) pcDNA 3.1Zeo(+)
3) ↓ 18 ex ↓ 1-8; 10; 11

Bam / Xba

Look like rect's.
Grow 18(1).

EXHIBIT
D95

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

6/5/97

RIPA - Infections:

Mock, ~~[illegible]~~, 1101/1107, 5U4, 101-1, 111-1, 734.1

E - early (9-15)
L - late (24-28)

293 E	293-TTF E	A549 E	293 L	293TTF L	A549 L
M - 1	7	13	19	25	31
1101/1107 - 2	8	14	20	26	32
5U4 - 3	9	15	21	27	33
101-1 - 4	10	16	22	28	34
111-1 - 5	11	17	23	29	35
734.1 - 6	12	18	24	30	36

A549	293
1101/1107 - 10 µl	1101/1107 - 100 µl
5U-4 - 100 µl	5U-4 - 1 ml
101-1 - 10 µl	101-1 - 100 µl
111-2 - ~~1~~50 µl	111-2 - 500 µl
734.1 - 1 ml	734.1 - 10 µl

- Viruses dosage (crude stocks of viruses)

6/10/97

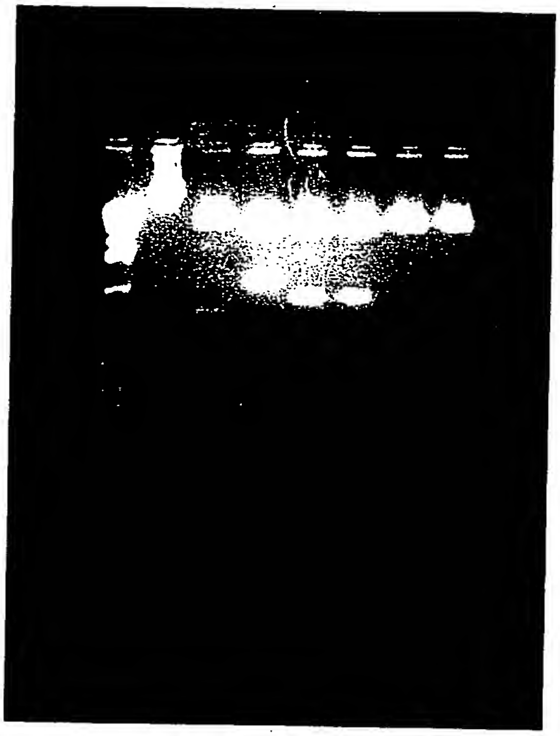
Restriction pattern for 181 maxiprep.

1) Ladder
2) 181/SpeI + PacI
3) 181/SpeI + XhoI.

Pattern is as expected.

EXHIBIT D96

6/25/97
Restriction digestion of pCI-14.7 (Terry).



1) ladder
2) pCI 14.7
3) — EcoRI
4) — BglII
5) — SpeI + XhoI
6) — SpeI + SalI
7) — BamHI + XhoI
8) — BamHI + SalI

7/1/97 Clonings 19ex, 20 ex:

19ex — vector p#30/SnaBI, insert 19ex1 { p181 Mfe Xba Klenow

19ex2 { p181 Mfe RV Klenow

20ex — vector pL2/PacI+T4, insert 20ex1 p181 MXKl

7/3 transformation DH5α.

20 V ~10
20 VL ~50
20ex1 ~100
20 ex11 ~30

19 V ~20
19 VL ~100
19 ex1 ~100
19ex11 ~100.

20ex2 p181 MRVKl

AMPAD 22-141 50 SHEETS 22-142 100 SHEETS 22-144 200 SHEETS

EXHIBIT
D97

DK

EXHIBIT
E

Dear Dan:

Attached is a list of primers that were ordered by Konstantin Doronin. The primers were named KD1, KD2, KD3, KD4, KD5, etc. (see Description/Sequences). These primers were used in studies on the ADP-overexpressing vectors, either for DNA sequencing or for amplification of certain sequences by the polymerase chain reaction (PCR). Also attached are Dr. Doronin's handwritten notes indicating the use of the primers. (These notes are not dated, but this list was begun when the primers were ordered). Please note that the primers were ordered on 9/26/96, 11/04/96, and 03/24/97.

Also included are some actual DNA sequence data using some of these primers to sequence some of the plasmids that were used to construct the ADP-overexpressing vectors. The dates of the sequencing runs are on the datasheets. Note that the first sequencing run was on November 19, 1996. Other runs were on November 25/26, 1996, March 20/21, 1997, April 21, 1997, May 12, 1997, and March 23/24, 1999.

Thanks,

Bill

Order Number		
Please refer to this No. on all inquires	Order Date	Page No.
473935 A	09/26/98	1 of 1

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DORONIN
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CUSTOMER REF	CUSTOMER P.O. NO	CUSTOMER CONTRACT NO/RELEASE NO	PLACED BY
MLR	5903851		FAX/DEBBIE HUMISTON

Line No	Cat No/ID No	Description/Sequence			Amount (OD)
1.0	10336-022 A3884G07	KD1 \ DORONIN CTACGAGAGAACCTCTCCGAG	1 Each	(G07)	19.3
2.0	10336-022 A3884G08	KD2 \ DORONIN GCCACAACTTATACTGTTTGC	1 Each	(G08)	15.5
3.0	10336-022 A3884G09	KD3 \ DORONIN TCAGCCCACGGTACTTAATTAACCCAAAAGGTGGAT	1 Each	(G09)	28.6
4.0	10336-022 A3884G10	KD4 \ DORONIN GCTCTAGAAGTCAGGCTTCCTGG	1 Each	(G10)	18.6
5.0	10336-022 A3884G11	KD5 \ DORONIN GCTCTAGATCTCATTTAATCATA	1 Each	(G11)	17.9
6.0	10336-022 A3884G12	KD6 \ DORONIN CCTTAATTAAAAGTCAGGCTTCCTGG	1 Each	(G12)	21.5
7.0	10336-022 A3884H01	KD7 \ DORONIN CCTTAATTAATCTCATTTAATCATA	1 Each	(H01)	20.3
8.0	10336-022 A3884H02	KD8 \ DORONIN CGCGTATACAGAAGATTTTTCCAG	1 Each	(H02)	21.7
9.0	10336-022 A3884H03	KD9 \ DORONIN CGCGTATACACTGCAGCAGGTGTG	1 Each	(H03)	20.5

EXHIBIT
B1

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EXHIBIT
E2

Primer 1:

Primer Name:	KD1	Primer Number:	A3884G07 (G07)
Researcher:	DORONIN	Primer Length:	21
Sequence (5' to 3'):	CTA CGA GAG AAC CTC TCC GAG		
Molecular Weight µg/µmole:	6740.2	µg per OD:	29.2
Millimolar Extinction Coefficient:	230.5	nmoles per OD:	4.3
Purity	**Deprotected**	**OD's**	**19.3**
Tm (1 M Na+)	**73**	**µg's***	**566**
Tm (50 mM Na+)	**51**	**nmoles**	**84**
% GC	**57**	**Coupling Eff.**	**99%**
Notes:			

Primer 2:

Primer Name:	KD2	Primer Number:	A3884G08 (G08)
Researcher:	DORONIN	Primer Length:	21
Sequence (5' to 3'):	GCC ACA ACT TAT ACT GTT TGC		
Molecular Weight µg/µmole:	6696.2	µg per OD:	30.2
Millimolar Extinction Coefficient:	221.4	nmoles per OD:	4.5
Purity	**Deprotected**	**OD's**	**15.5**
Tm (1 M Na+)	**67**	**µg's***	**471**
Tm (50 mM Na+)	**45**	**nmoles**	**70**
% GC	**42**	**Coupling Eff.**	**99%**
Notes:			

Primer 3:

Primer Name:	KD3	Primer Number:	A3884G09 (G09)
Researcher:	DORONIN	Primer Length:	36
Sequence (5' to 3'):	TCA GCC CAC GGT ACT TAA TTA ACC CAA AAG GTG GAT		
Molecular Weight µg/µmole:	11632.2	µg per OD:	28.5
Millimolar Extinction Coefficient:	407.2	nmoles per OD:	2.4
Purity	**Deprotected**	**OD's**	**28.6**
Tm (1 M Na+)	**81**	**µg's***	**817**
Tm (50 mM Na+)	**59**	**nmoles**	**70**
% GC	**44**	**Coupling Eff.**	**99%**
Notes:			

* -See Note about Quantities in Supporting Information.

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Primer 4:

Primer Name: KD4
Researcher: DORONIN
Sequence (5' to 3'):GCT CTA GAA GTC AGG CTT CCT GG
Molecular Weight µg/µmole: 7429.6
Millimolar Extinction Coefficient: 244.0

Purity	**Deprotected**
Tm (1 M Na+)	**75**
Tm (50 mM Na+)	**54**
% GC	**56**

Notes:

Primer Number: A3884G10 (G10)
Primer Length: 23

µg per OD:	30.4
nmoles per OD:	4.1
OD's	**18.6**
µg's*	**568**
nmoles	**76**
Coupling Eff.	**99%**

Primer 5:

Primer Name: KD5
Researcher: DORONIN
Sequence (5' to 3'):GCT CTA GAT CTC ATT TAA TCA TA
Molecular Weight µg/µmole: 7346.6
Millimolar Extinction Coefficient: 251.4

Purity	**Deprotected**
Tm (1 M Na+)	**65**
Tm (50 mM Na+)	**43**
% GC	**30**

Notes:

Primer Number: A3884G11 (G11)
Primer Length: 23

µg per OD:	29.2
nmoles per OD:	3.9
OD's	**17.9**
µg's*	**523**
nmoles	**71**
Coupling Eff.	**99%**

Primer 6:

Primer Name: KD6
Researcher: DORONIN
Sequence (5' to 3'):CCT TAA TTA AAA GTC AGG CTT CCT GG
Molecular Weight µg/µmole: 8370.2
Millimolar Extinction Coefficient: 284.9

Purity	**Deprotected**
Tm (1 M Na+)	**73**
Tm (50 mM Na+)	**51**
% GC	**42**

Notes:

Primer Number: A3884G12 (G12)
Primer Length: 26

µg per OD:	29.3
nmoles per OD:	3.5
OD's	**21.5**
µg's*	**634**
nmoles	**75**
Coupling Eff.	**99%**

* -See Note about Quantities in Supporting Information.

EXHIBIT
B3

LIFE TECHNOLOGIES

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Primer 7:

Primer Name: KD7
Researcher: DORONIN
S quence (5' to 3'):CCT TAA TTA ATC TCA TTT AAT CAT A
Molecular Weight µg/µmole: 7957.0
Millimolar Extinction Coefficient: 277.0

Purity	**Deprotected**
Tm (1 M Na+)	**63**
Tm (50 mM Na+)	**41**
% GC	**20**

Notes:

Primer Number: A3884H01 (H01)
Primer Length: 25

µg per OD: 28.7
nmoles per OD: 3.6

OD's	**20.3**
µg's*	**585**
nmoles	**73**
Coupling Eff.	**99%**

Primer 8:

Primer Name: KD8
Researcher: DORONIN
Sequence (5' to 3'):CGC GTA TAC AGA AGA TTT TTC CAG
Molecular Weight µg/µmole: 7742.8
Millimolar Extinction Coefficient: 268.2

Purity	**Deprotected**
Tm (1 M Na+)	**70**
Tm (50 mM Na+)	**49**
% GC	**41**

Notes:

Primer Number: A3884H02 (H02)
Primer Length: 24

µg per OD: 28.8
nmoles per OD: 3.7

OD's	**21.7**
µg's*	**628**
nmoles	**81**
Coupling Eff.	**99%**

Primer 9:

Primer Name: KD9
Researcher: DORONIN
Sequence (5' to 3'):CGC GTA TAC ACT GCA GCA GGT GTG
Molecular Weight µg/µmole: 7784.8
Millimolar Extinction Coefficient: 261.8

Purity	**Deprotected**
Tm (1 M Na+)	**77**
Tm (50 mM Na+)	**56**
% GC	**58**

N tes:

Primer Number: A3884H03 (H03)
Primer Length: 24

µg per OD: 29.7
nmoles per OD: 3.8

OD's	**20.5**
µg's*	**609**
nmoles	**78**
Coupling Eff.	**99%**

* -See Note about Quantities in Supporting Information.

EXHIBIT
E4

LIFE TECHNOLOGIES

Order Number		
Please refer to this No. on all inquires	Order Date	Page No.
522393 A	11/04/96	1 of 1

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CUSTOMER REP	CUSTOMER P.O. NO.	CUSTOMER CONTRACT NO/RELEASE NO	PLACED BY
CEH	S903851		FAX/DEBBIE HUMISTON

Line No	Cat No/ID No	Description/Sequence			Amount (OD)
1.0	10336-022 A4379B02	KD 10 \ DORANIN ACACGGCACCAGCTCAATCAG	1 Each	(B02)	12.6
2.0	10336-022 A4379B03	KD 11 \ DORANIN CGGAGTAACTTGTATGTGTTG	1 Each	(B03)	12.5

EXHIBIT
53

ST LOUIS UNIV SCHOOL OF	
Order Number:	522393A
Order Date:	11/04/96

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Primer 1:

Primer Name: KD 10
Researcher: DORANIN
Sequence (5' to 3'):ACA CGG CAC CAG CTC AAT CAG
Molecular Weight µg/µmole: 6709.2
Millimolar Extinction Coefficient: 232.1

Purity	Deprotected
Tm (1 M Na+)	73
Tm (50 mM Na+)	51
% GC	57

Notes:

Primer Number: A4379B02 (B02)
Primer Length: 21

µg per OD:	28.9
nmoles per OD:	4.3
OD's	12.6
µg's*	366
nmoles	54
Coupling Eff.	98%

Primer 2:

Primer Name: KD 11
Researcher: DORANIN
Sequence (5' to 3'):CGG AGT AAC TTG TAT GTG TTG
Molecular Weight µg/µmole: 6847.2
Millimolar Extinction Coefficient: 233.0

Purity	Deprotected
Tm (1 M Na+)	67
Tm (50 mM Na+)	45
% GC	42

Notes:

Primer Number: A4379B03 (B03)
Primer Length: 21

µg per OD:	29.3
nmoles per OD:	4.2
OD's	12.5
µg's*	369
nmoles	53
Coupling Eff.	98%

* -See Note about Quantities in Supporting Information.

EXHIBIT
56

LIFE TECHNOLOGIES

Order Number		
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409867 A	03/24/97	1 of 1

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000127752

CUSTOMER REP	CUSTOMER P.O. NO	CUSTOMER CONTRACT NO./RELEASE NO	PLACED BY
CD	S903850		DEBBIE HUMISTON

Line No.	Cat No./ID No.	Description/Sequence			Amount (OD)
1.0	10336-022 A6280D10	KD12 \ DORONIN CCTTAATTAATCTAGAGATCTTATTCCCTTT	1 Each	(D10)	16.4
2.0	10336-022 A6280D11	KD14 \ DORONIN GGGGTACGAAGCCATCTGCAACAACAT	1 Each	(D11)	14.7
3.0	10336-022 A6280D12	DK15 \ DORONIN CCTTAATTAATCTAGAGTCAGTTAGCCTCCCC	1 Each	(D12)	13.7
4.0	10336-022 A6280E01	KD16 \ DORONIN CGCGCGTATACACTTCCCATTTTAAG	1 Each	(E01)	14.6
5.0	10336-022 A6280E02	KD17 \ DORONIN GCTCTAGACATCATCAATAATAT	1 Each	(E02)	12.9

EXHIBIT
F7

ST LOUIS UNIV SCHOOL OF	
Order Number:	409867A
Order Date:	03/24/97

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Primer 4:

Primer Name:	KD16	Primer Number:	A6280E01 (E01)
Researcher:	DORONIN	Primer Length:	26
Sequence (5' to 3'):	CGC GCG TAT ACA CTT CCC ATT TTA AG		
Molecular Weight µg/µmole:	8306.2	µg per OD:	30.4
Millimolar Extinction Coefficient:	272.6	nmoles per OD:	3.6
Purity	**Deprotected**	**OD's**	**14.6**
Tm (1 M Na+)	**74**	**µg's***	**444**
Tm (50 mM Na+)	**53**	**nmoles**	**53**
% GC	**46**	**Coupling Eff.**	**99%**
N tes:			

Primer 5:

Primer Name:	KD17	Primer Number:	A6280E02 (E02)
Researcher:	DORONIN	Primer Length:	23
Sequence (5' to 3'):	GCT CTA GAC ATC ATC AAT AAT AT		
Molecular Weight µg/µmole:	7364.6	µg per OD:	27.9
Millimolar Extinction Coefficient:	263.4	nmoles per OD:	3.8
Purity	**Deprotected**	**OD's**	**12.9**
Tm (1 M Na+)	**65**	**µg's***	**361**
Tm (50 mM Na+)	**43**	**nmoles**	**49**
% GC	**30**	**Coupling Eff.**	**99%**
Notes:			

* -See Note about Quantities in Supporting Information.

LIFE TECHNOLOGIES.

EXHIBIT
D-8

Primers:

1) KD1 - CTA'CGA'GAG'AAC'CTC'TCC'GAG'

Comment - primer from Ad5 sequence ~50 nt left from SauI site - for PCR mutation of gp19K (E3) - Left primer.

2) KD2 - GCC'ACA'ACT'TAT'ACT'GTT'TGC

Comment - primer from Ad5 sequence ~50 nt right from Bst1107I site - for PCR mutation of gp19K (E3) - right primer.

3) KD3 - TCA'GCC'CAC'GGT'AC|T TAA T TAA|CC'CAA'AAG'GTG'GA

Comment - middle primer for mutation of gp19K E3 protein gene. inserting PacI site with TAA stop codon to the ORF destroying KpnI site simultaneously, to be used 1 with KD2, then product used as primer with KD1.

4) KD4 - GC|TCT'AGA|'AGT'CAG'GCT'TCC'TGG

Comment - left primer for PCR of 11.6K E3 with XbaI site.

5) KD5 - GC|TCT'AGA|'TCT'CAT'TTA'ATC'ATA

Comment - right primer for PCR of 11.6K E3 with XbaI site.

6) KD6 - CC|TTAATTAA|GT'CAG'GCT'TCC'TGG

Comment - left primer for PCR of 11.6K E3 with PacI site

AA

7) KD7 - CC|TTAATTAA|TCT'CAT'TTA'ATC'ATA

Comment - right primer for PCR of 11.6K E3 with PacI site.

8) KD8 CGC|GTATAC|'AGA'AGA'TTT'TTC'CAG'

upstream primer with Bst1107I site for PCR of SP-B 500 promoter.

9) KD9 CGC|GTATAC|ACT'GCA'GCA'GGT'GTG

downstream primer with Bst1107I site for PCR of SP-B 500 promoter.

22-141 50 SHEETS
22-142 100 SHEETS
22-144 200 SHEETS
AMPAD

EXHIBIT
59

Primers for sequencing E4 substitutions ; promoter.

10) KD 10: ACA'CGG'CAC'CAG'CTC'AAT'CAG' (left)

11) KD 11: CGG'AGT'AAC'TTG'TAT'GTG'TTG (right)

Primers for TK insert into Pac and Xba sites. (double PcR, 12+14, then product with 15 on Ad template).

12) KD 12

CC

TTAATTAA TCTAGA GAT'CTT'ATT'CCC'TTT

Primer with Pac and Xba sites + Ad seq 30818 → upstream primer.

14) KD 14 ← of tk

GGG'GTA'CGA'AGC CAT 'CTG'CAA'CAA'CAT'

Primer left half from 5' tk orf, right half - from Ad ~ 31080 f
⇒ beginning of fiber gene.

KD 15 to prevent methylation.

15) 15 CC TTAATTAA TCTAGA G TCA'GTT'AGC'CTC'CC

~~of tk~~ .

(3)

Primers to PCR Ad5 right ITR (Ad. 35773-35935) with flanking bst1107I and XbaI sites. respectively.

KD 16

16) CGCCC GTATAC ACT'TCC'CAT'TTT'AAG

KD 17

17) GC TCTAGA CAT'CAT'CAA'TAA'TAT

KD 18 - Direct - GTA-GAG-TTT-TCT-CCT-CCG } for PCR and sequence of 1101/1107 dl.

KD 19 - Reverse - CCC-TCT-TCA-TCC-TCG-TCG

KD 20 - TCT,ACT,TTA,ACC,CCT,TCT,CGG (E3 direct to ~~CUX~~ dl)

KD 21 - cgg,agg,tgt,tat,tac,cga,aga (E1A ~~1101~~ dl) direct

KD 22 - TGA,CGT,AAC,GGT,TAA,AGT,CCA (from E4 prom dl) direct

FROM :SAINT LOUIS UNIVERSITY MMI FAX NO. :3147733403 Nov. 26 2002 06:03PM P12

ABI PRISM
Model 310
Version 2.1.2

sex clone 1
primer 1(rc) 1

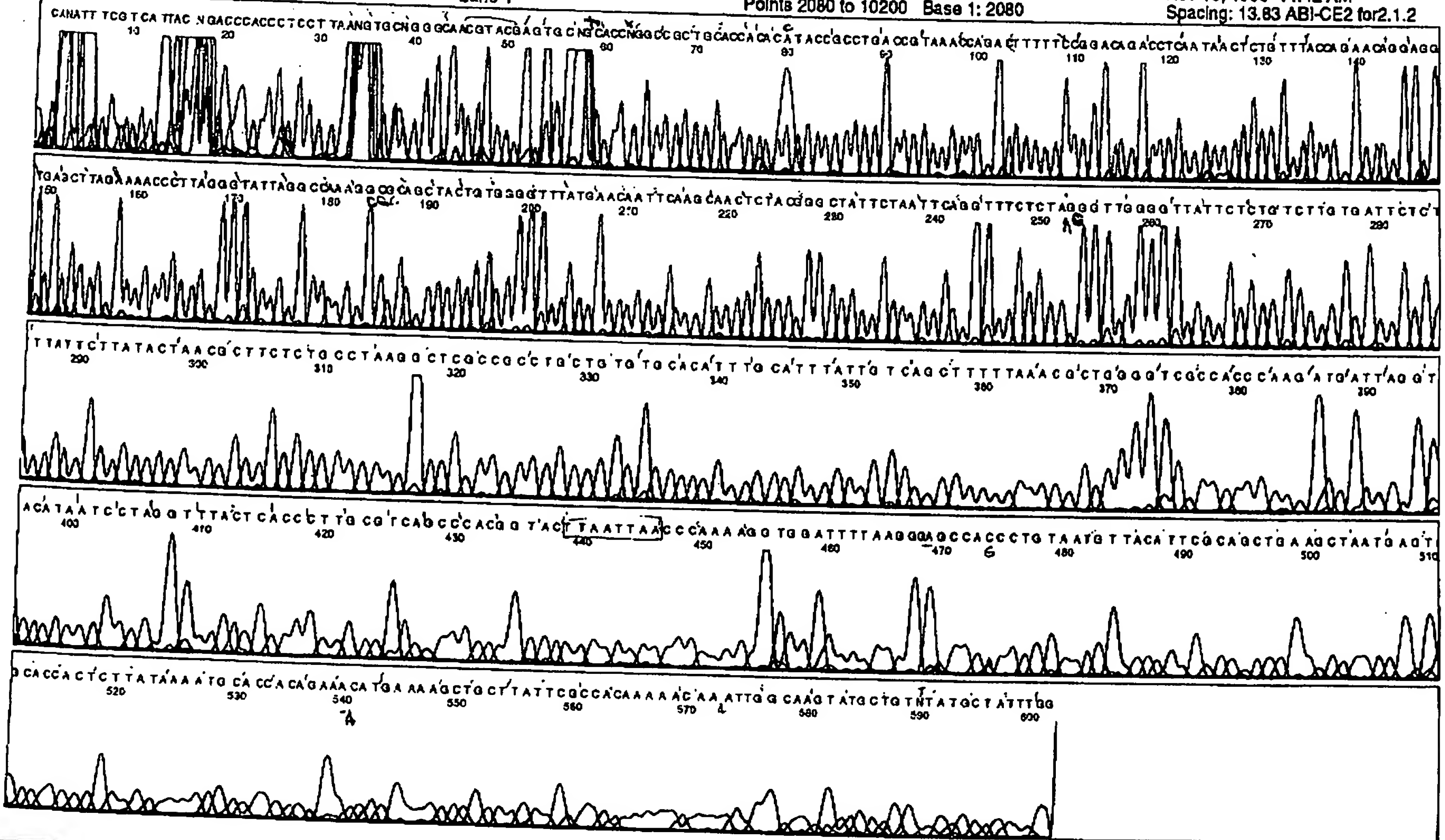
A1-KD

KD
Lane 1

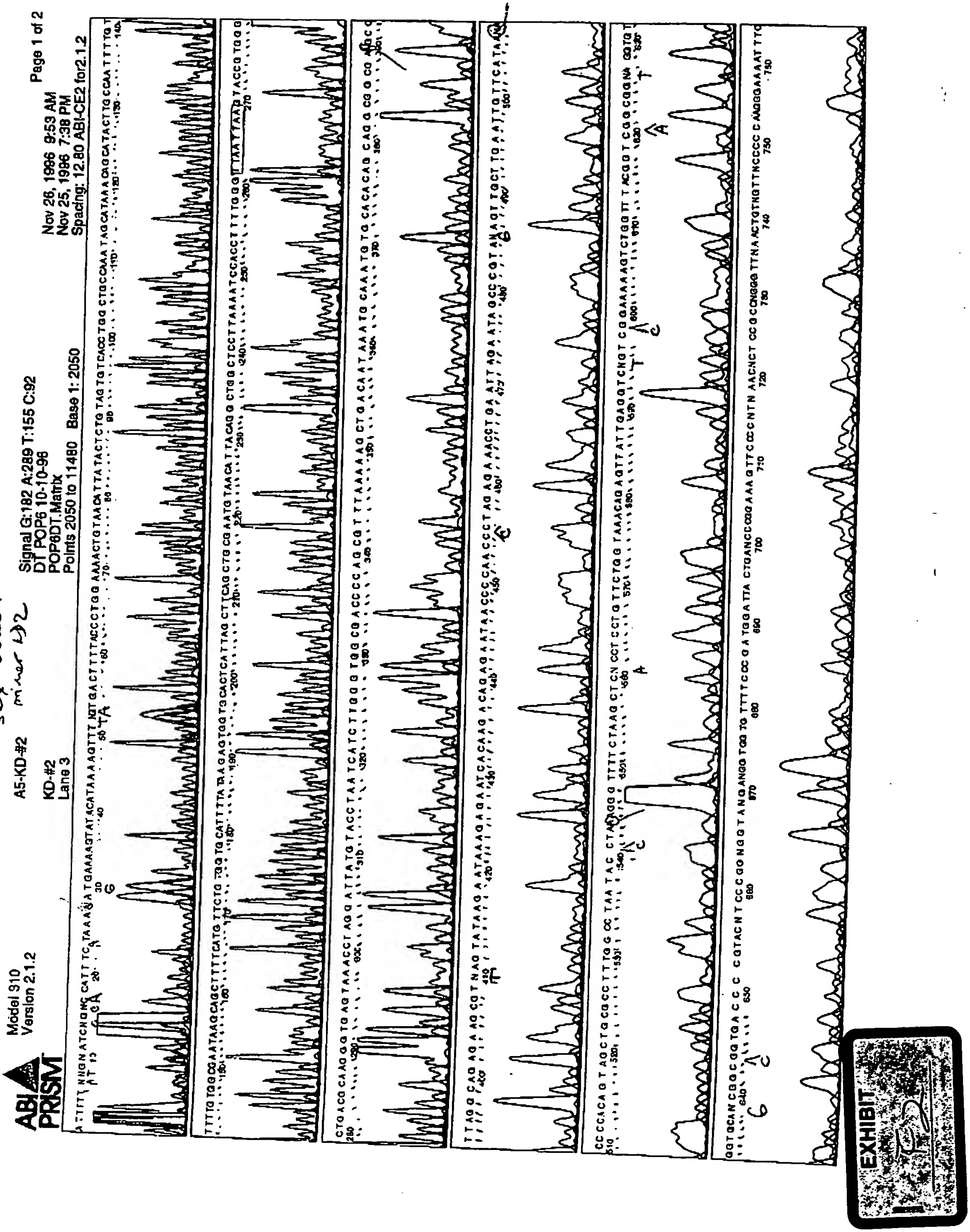
Signal G:340 A:481 T:305 C:253
DT POP6 10-10-96
POP6DT.Matrix
Points 2080 to 10200 Base 1: 2080

Page 1 of 1

Nov 19, 1996 2:40 PM
Nov 19, 1996 11:42 AM
Spacing: 13.83 ABI-CE2 for2.1.2



EXHIBIT


ABI PRISM
Model 310
Version 2.1.2
A5-KD-#2
KD-#2
Lane 3
Signal G:182 A:289 T:155 C:92
DT POP6 10-10-96
POP6DT.Matrix
Points 2050 to 11480 Base 1: 2050
Nov 26, 1996 9:53 AM
Nov 25, 1996 7:38 PM
Spacing: 12.80 ABI-CE2 for2.1.2
Page 1 of 2
EXHIBIT

ABI PRISM

Model 310
Version 2.1.2

A7-KD-#3

KD-#3
Lane 4

gax, clone 23, primer KD10

Signal G:303 A:375 T:250 C:219
DT POP6 10-10-96
POP6DT.Matrix
Points 2040 to 11480 Base 1: 2040

Page 1 of 1

Nov 26, 1996 9:53 AM
Nov 25, 1996 10:28 PM
Spacing: 13.29 ABI-CE2 for2.1.2

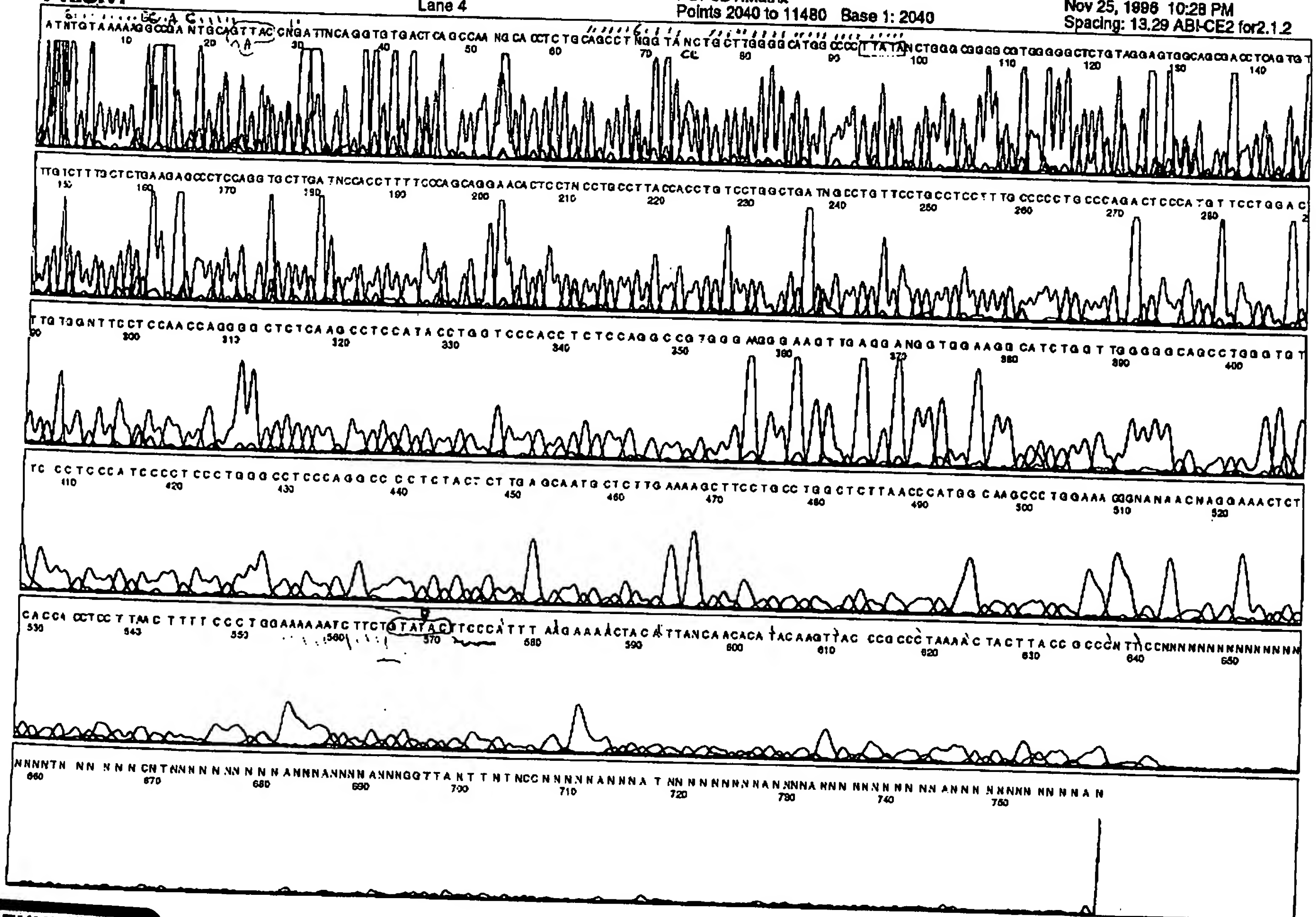


EXHIBIT
F3

2 ex, clone 23, primer KD II

ABI PRISM

Model 310
Version 2.1.2

A9-KD-#4

KD-#4
Lane 5

Signal G:499 A:664 T:279 C:205
DT POP6 10-10-96
POP6DT.Matrix
Points 2050 to 11480 Base 1: 2050

Nov 26, 1996 9:53 AM
Nov 26, 1996 1:18 AM
Spacing: 12.96 ABI-CE2 for2.1.2

Page 1 of 1

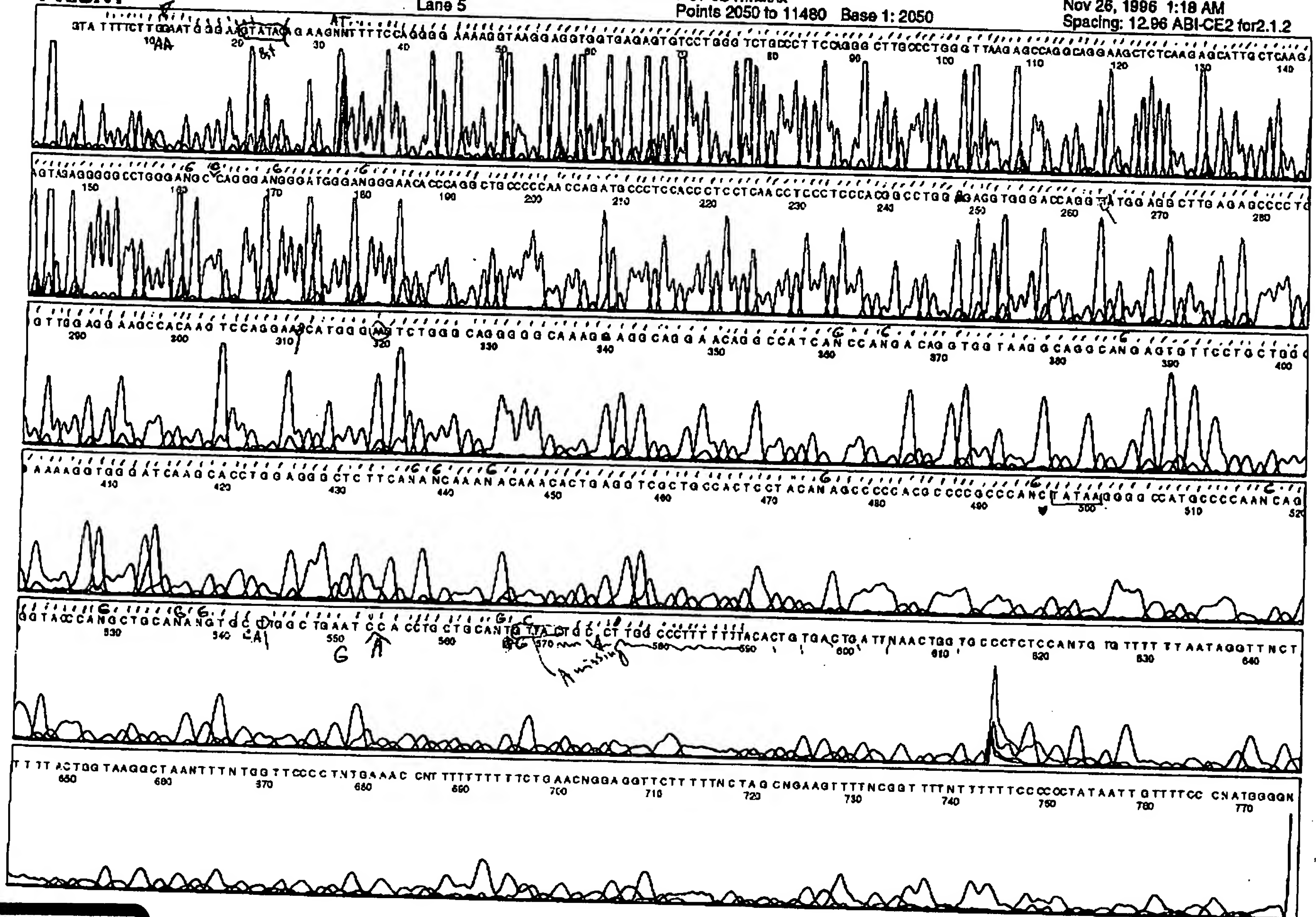


EXHIBIT F4

1 ex, clone 23, primer K910.

ABI PRISM

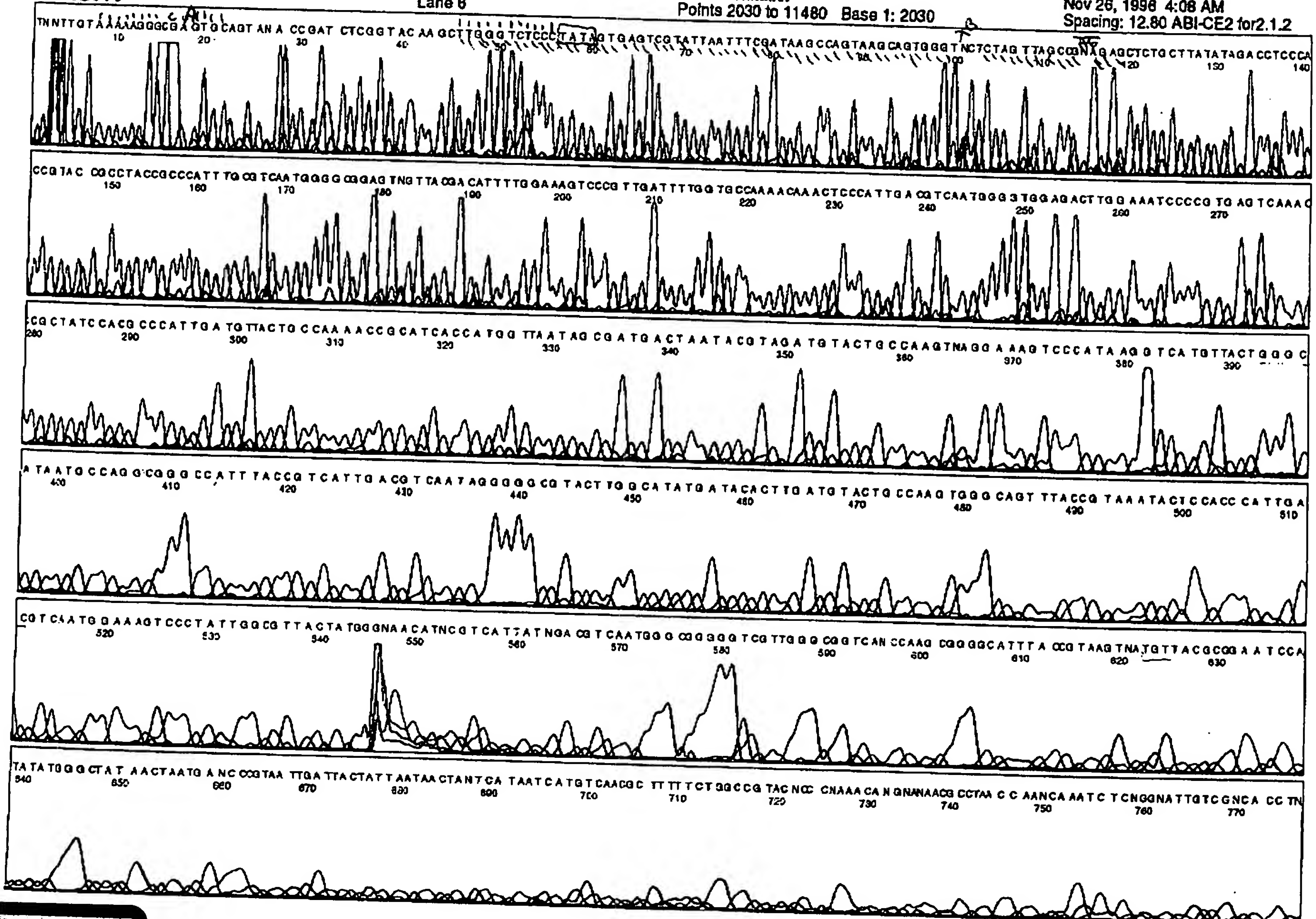
Model 310
Version 2.1.2

A11-KD-#5

KD-#5
Lane 6

Signal G:339 A:578 T:329 C:216
DT POP6 10-10-96
POP6DT.Matrix
Points 2030 to 11480 Base 1: 2030

Nov 26, 1996 9:53 AM
Nov 26, 1996 4:08 AM
Spacing: 12.80 ABI-CE2 for2.1.2



EXHIBIT

1 ex, clone 23, primer (c) 11.

ABI PRISM

Model 310
Version 2.1.2

B2-KD-#6

KD-#6
Lane 7

Signal G:343 A:526 T:302 C:163
DT POP6 10-10-96
POP6DT.Matrix
Points 2040 to 11480 Base 1: 2040

Nov 26, 1996 9:54 AM
Nov 26, 1996 6:57 AM
Spacing: 12.96 ABI-CE2 for2.1.2

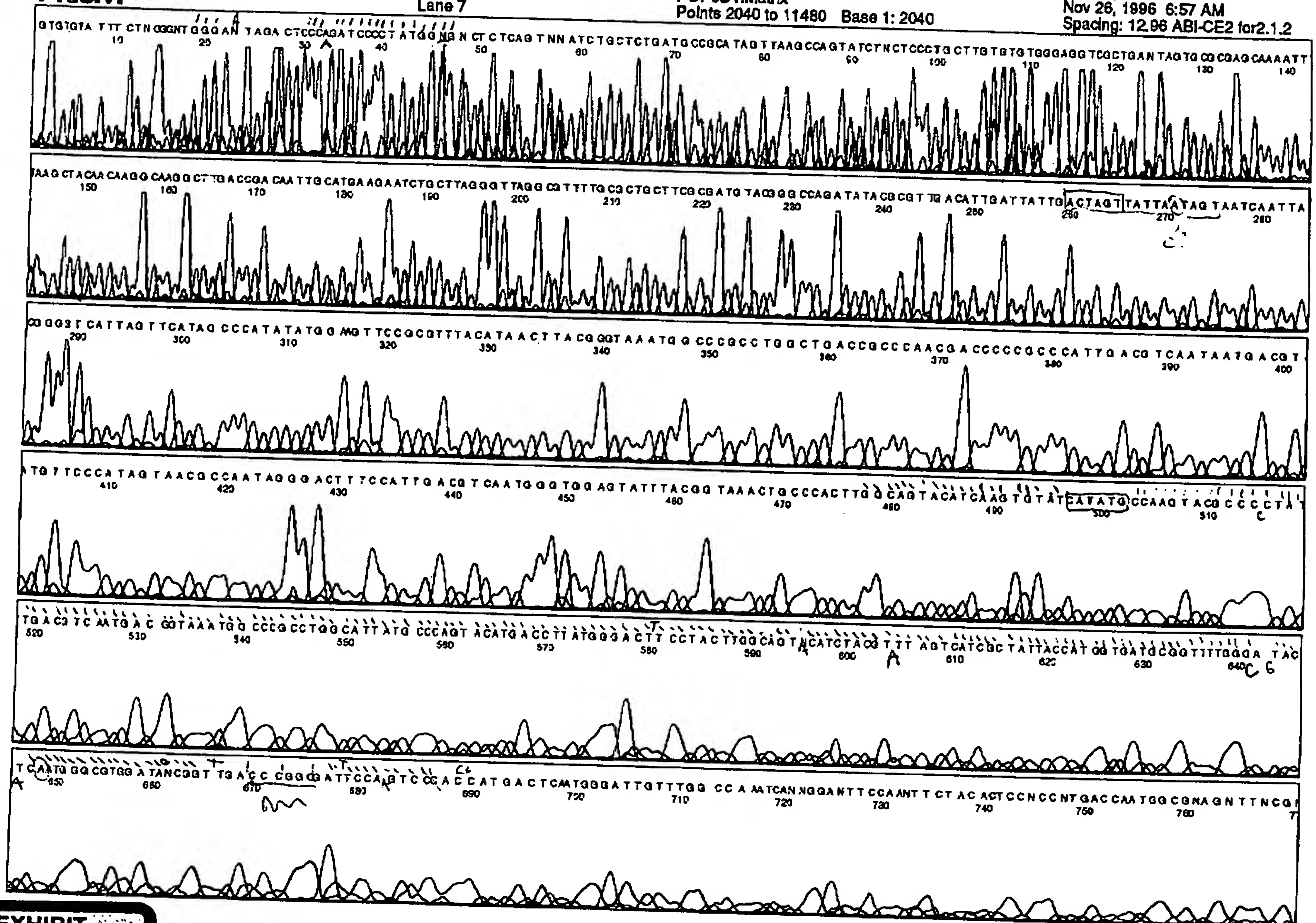


EXHIBIT
F6

Sex, clone 4, primer ...

ABI PRISM

Model 310
Version 2.1.2

B4-KD-#7

KD-#7
Lane 8

Signal G:176 A:383 T:218 C:160
DT POP6 10-10-96
POP6DT.Matrix
Points 2050 to 11480 Base 1: 2050

Nov 26, 1996 1:21 PM
Nov 26, 1996 9:47 AM
Spacing: 14.02 ABI-CE2 1or2.1.2

Page 1 of 1

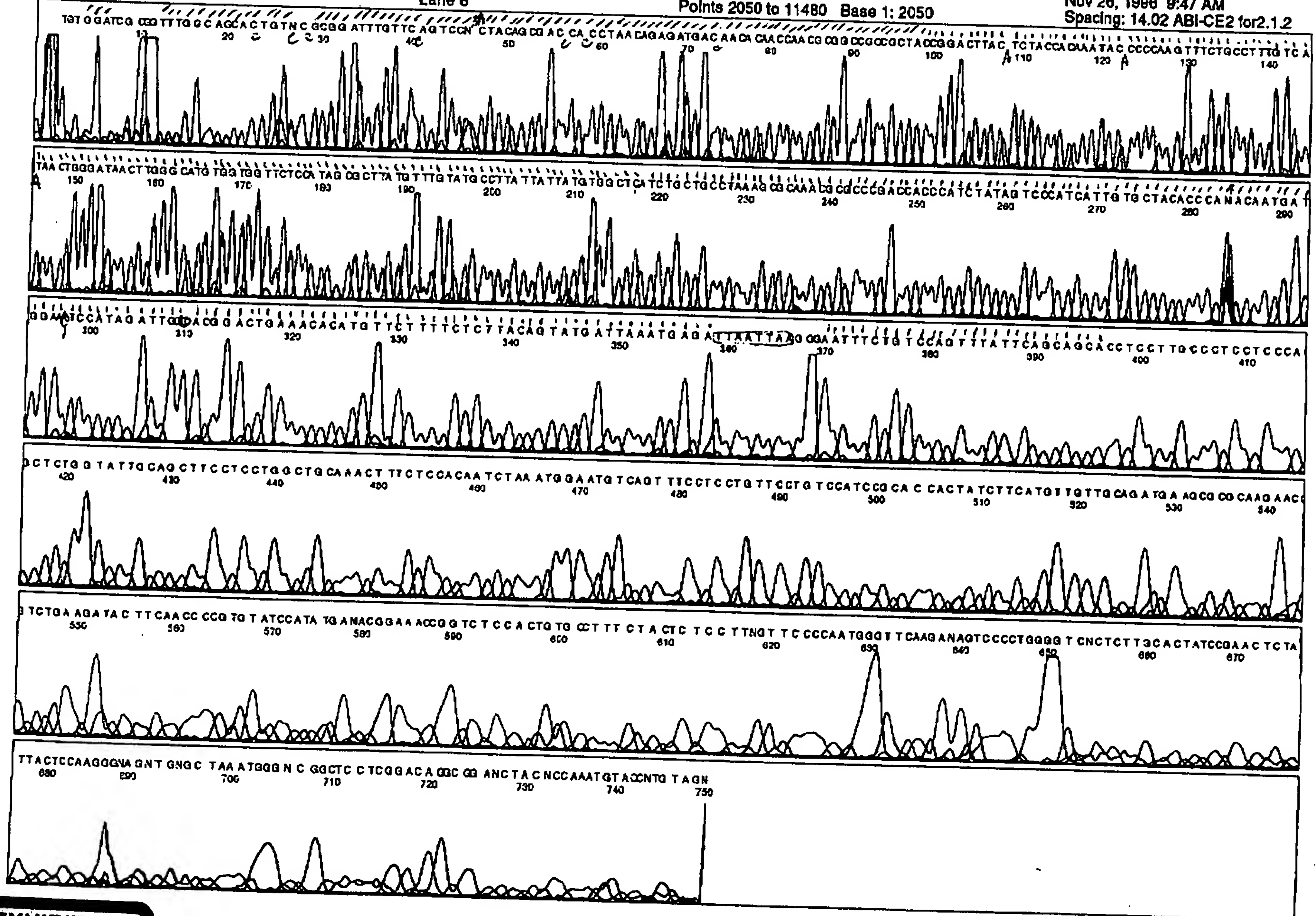


EXHIBIT
F7

ABI PRISM

Model 310
Version 2.1.2

B6-KD-#8

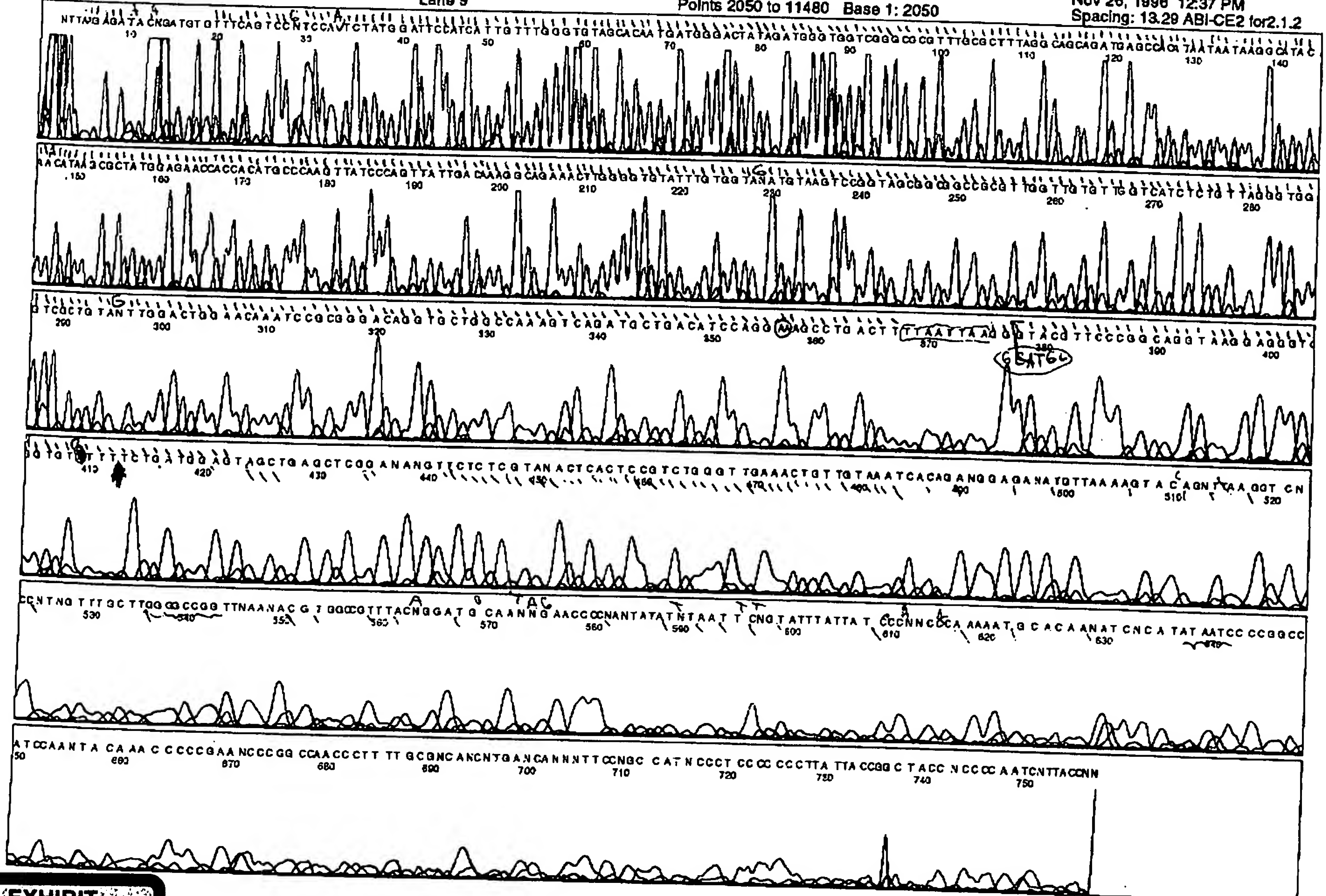
KD-#8
Lane 9

5ex, clone 4, primer 7

Signal G:192 A:458 T:261 C:140
DT POP6 10-10-96
POP6DT.Matrix
Points 2050 to 11480 Base 1: 2050

Nov 26, 1996 3:28 PM
Nov 26, 1996 12:37 PM
Spacing: 13.29 ABI-CE2 for2.1.2

Page 1 of 1



EXHIBIT

ABI PRISM™

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

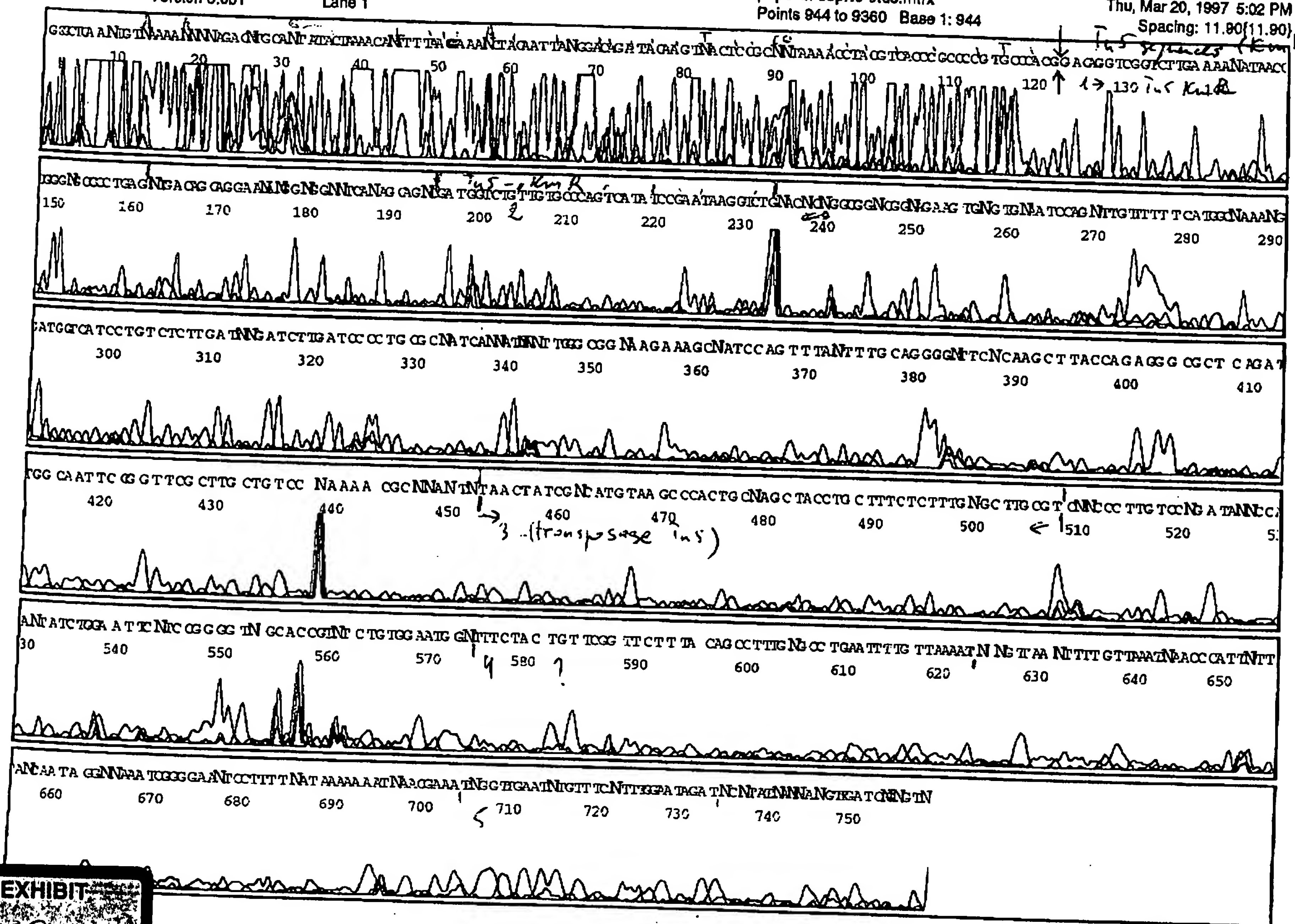
A1-Wold-9

Wold-9
Lane 1

(p21, primer kj10)

Signal G:151 A:256 T:158 C:157
DT POP6
pop6 dt-seprte-stds.mtrx
Points 944 to 9360 Base 1: 944

Page 1 of 1
Thu, Mar 20, 1997 7:47 PM
Thu, Mar 20, 1997 5:02 PM
Spacing: 11.90(11.90)



ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

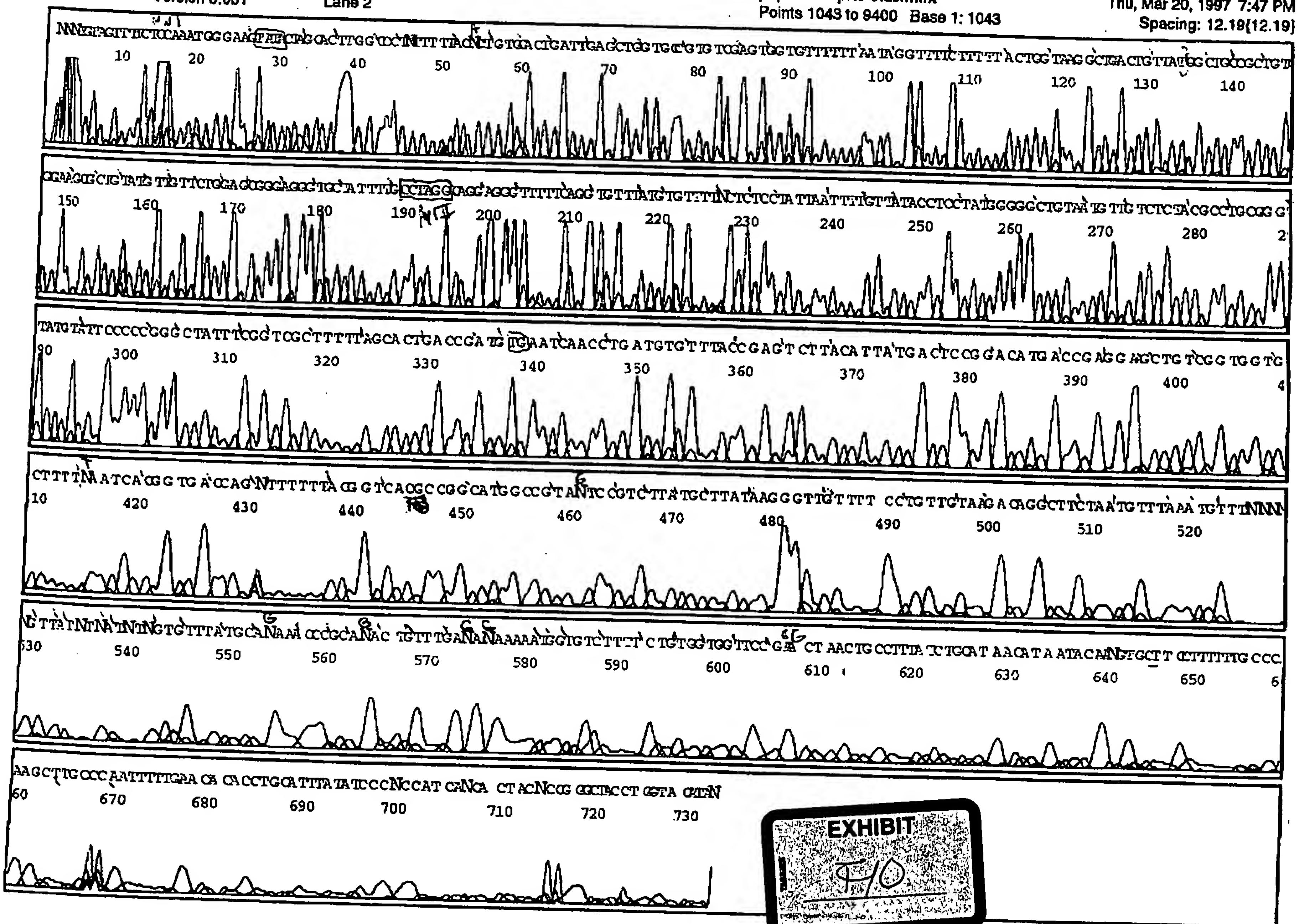
A3-Wold-10

Wold-10
Lane 2

ny10
(p21, primer k) 11)

Signal G:639 A:658 T:543 C:294
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1043 to 9400 Base 1: 1043

Page 1 of 1
Thu, Mar 20, 1997 10:22 PM
Thu, Mar 20, 1997 7:47 PM
Spacing: 12.19(12.19)



EXHIBIT

ABI PRISM™

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

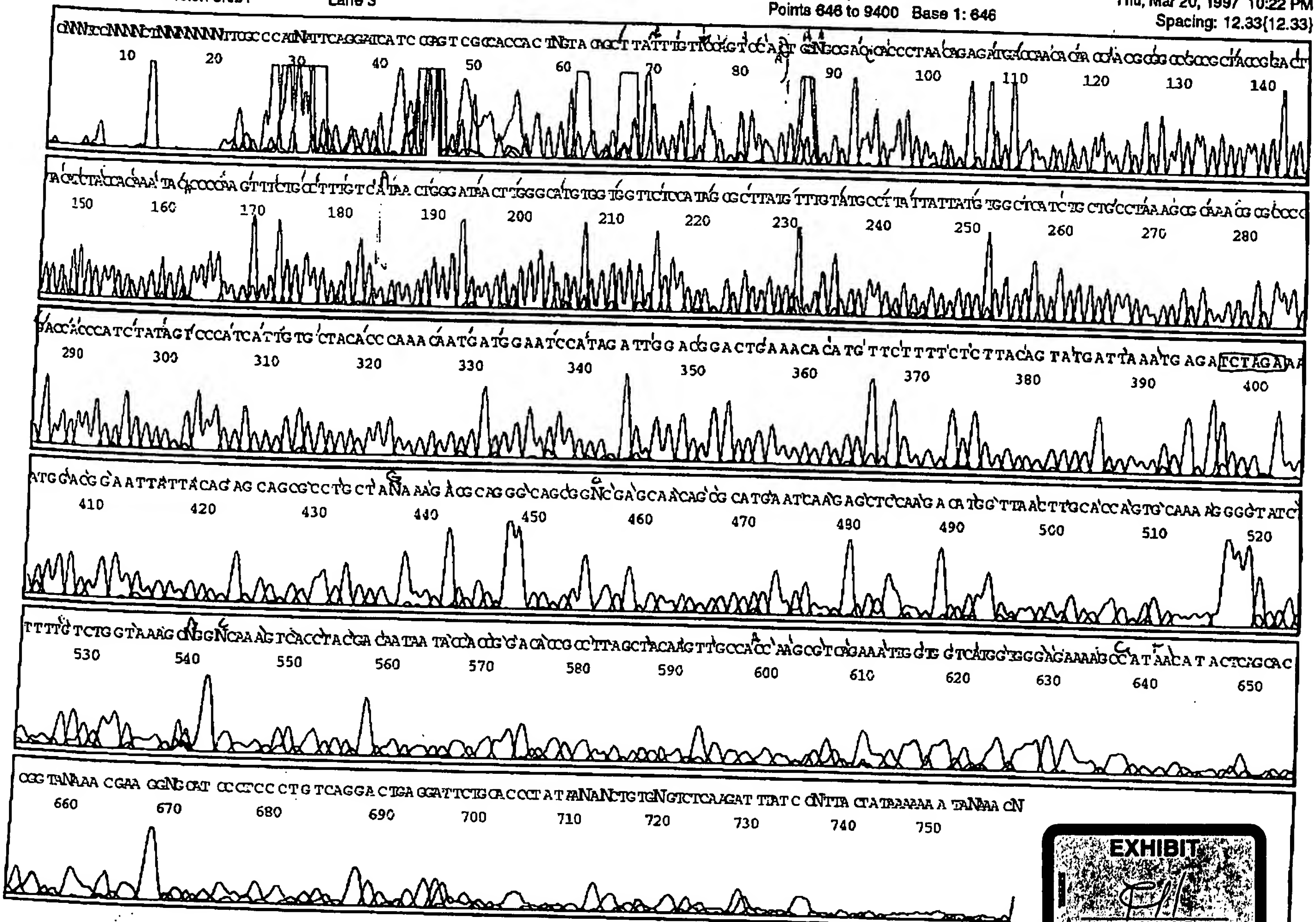
A5-Wold-11

Wold-11
Lane 3

kd 11
(pIII, primer kd4)

Signal G:278 A:413 T:211 C:190
DT POP6
pop6 dt-seprte-stds.mtrx
Points 646 to 9400 Base 1: 646

Page 1 of 1
Fri, Mar 21, 1997 12:57 AM
Thu, Mar 20, 1997 10:22 PM
Spacing: 12.33(12.33)



EXHIBIT

ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

A7-Wold-12

Wold-12
Lane 4

W) 12
(p111, primer W)5)

Signal G:251 A:255 T:160 C:128
DT POP6
pop6 dt-seprte-stds.mtrx
Points 657 to 9400 Base 1: 657

Page 1 of 1
Fri, Mar 21, 1997 3:32 AM
Fri, Mar 21, 1997 12:57 AM
Spacing: 12.33(12.33)

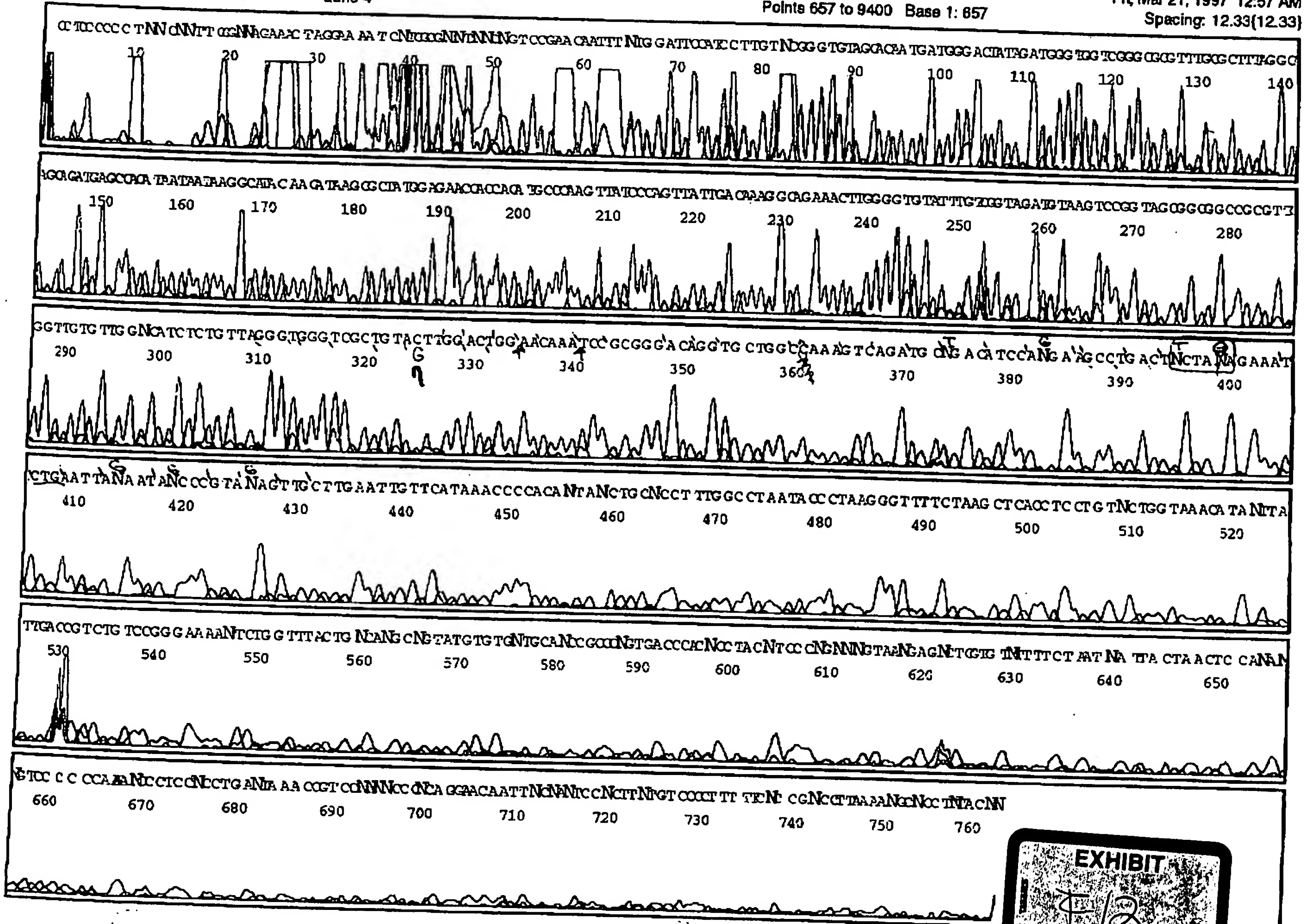


EXHIBIT
E12

ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

A1-Kostya/Wold-14

Kostya/Wold-14
Lane 1

pBHG11,
minus KD10.

Signal G:71 A:84 T:76 C:57
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1106 to 9400 Base 1: 1106

Page 1 of 1
Mon, Apr 21, 1997 12:49 PM
Mon, Apr 21, 1997 9:22 AM
Spacing: 12.64(12.64)

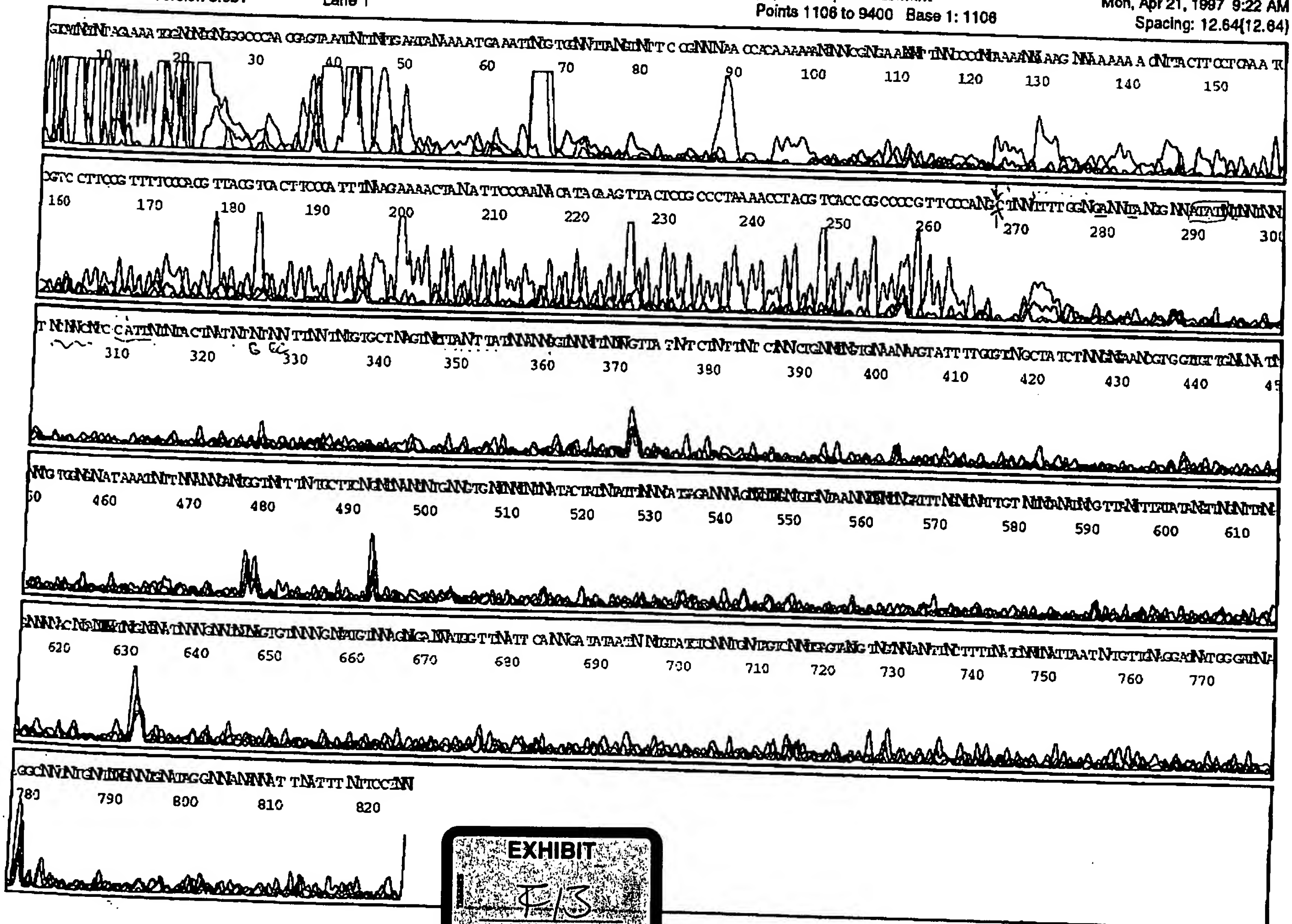


EXHIBIT
E13

ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

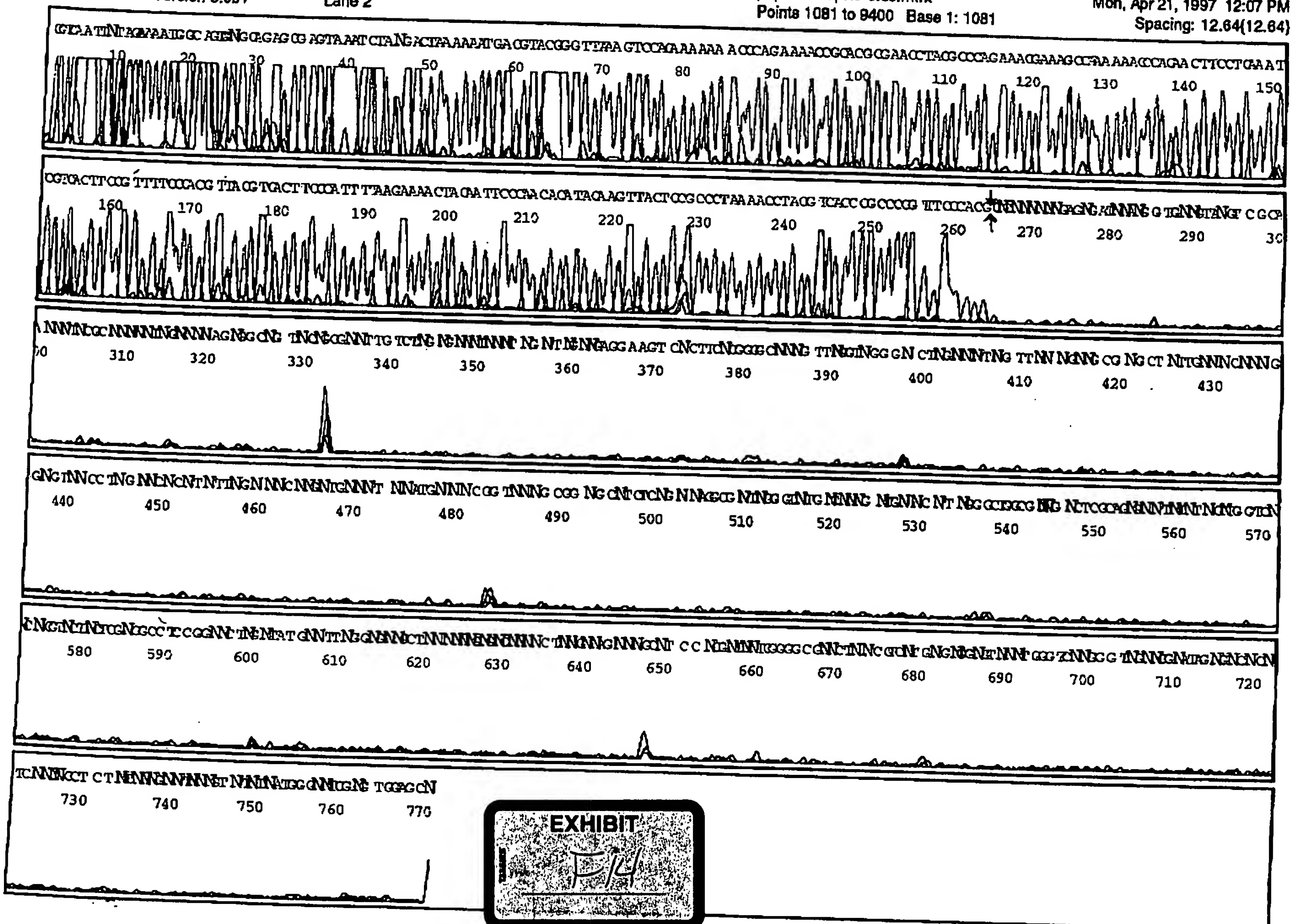
A3-Kostya/wold-15

Kostya/wold-15
Lane 2

pBUG10,
primer ws10.

Signal G:151 A:330 T:175 C:161
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1081 to 9400 Base 1: 1081

Page 1 of 1
Mon, Apr 21, 1997 2:42 PM
Mon, Apr 21, 1997 12:07 PM
Spacing: 12.64(12.64)



ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

A5-Kostya/wold-16

Kostya/wold-16
Lane 3

I-Ppo's Ecoli large plasmid, primer 16.

Signal G:99 A:145 T:99 C:92
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1079 to 9400 Base 1: 1079

Page 1 of 1
Mon, Apr 21, 1997 5:18 PM
Mon, Apr 21, 1997 2:42 PM
Spacing: 12.48(12.48)

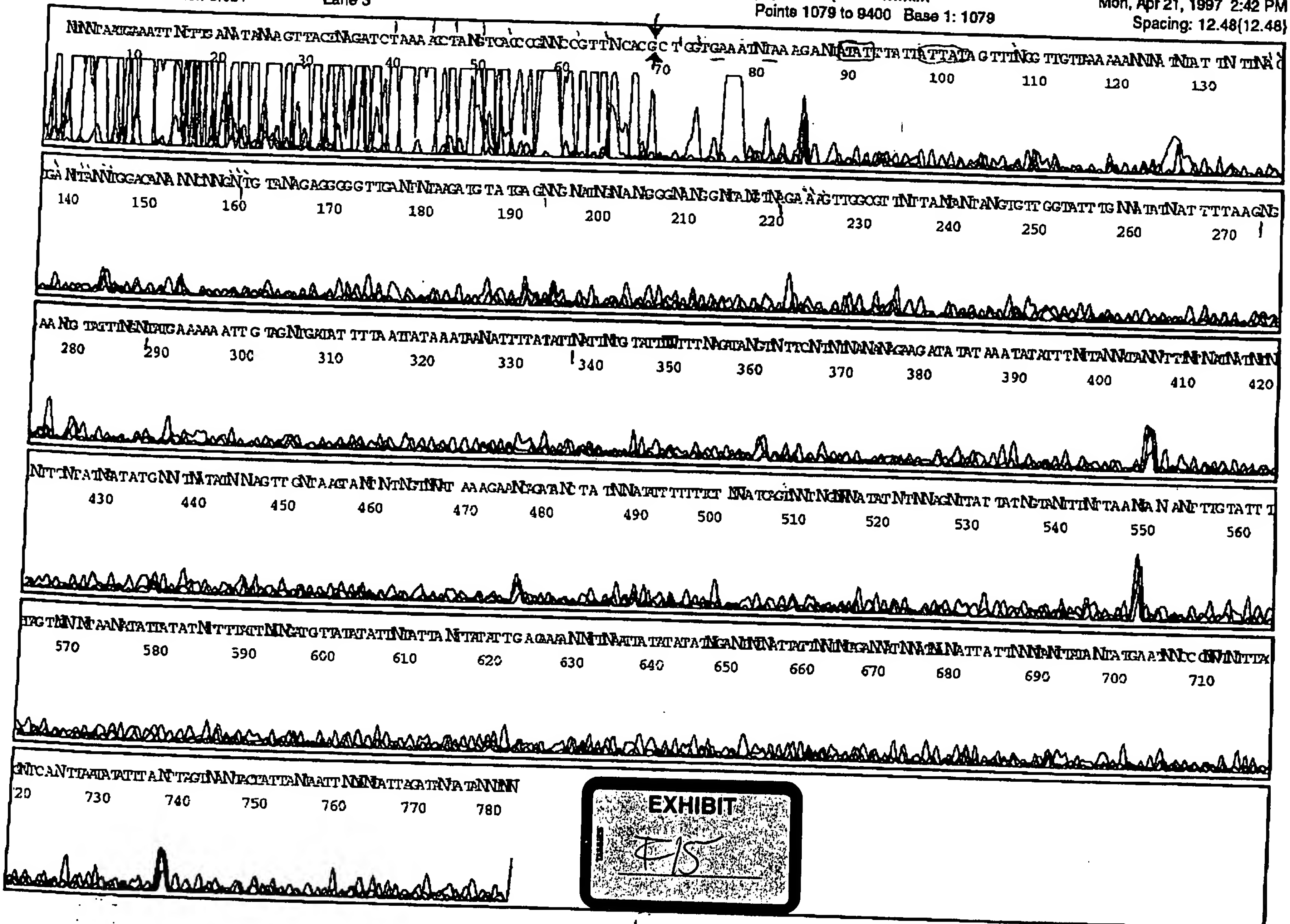


EXHIBIT
F-15

ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

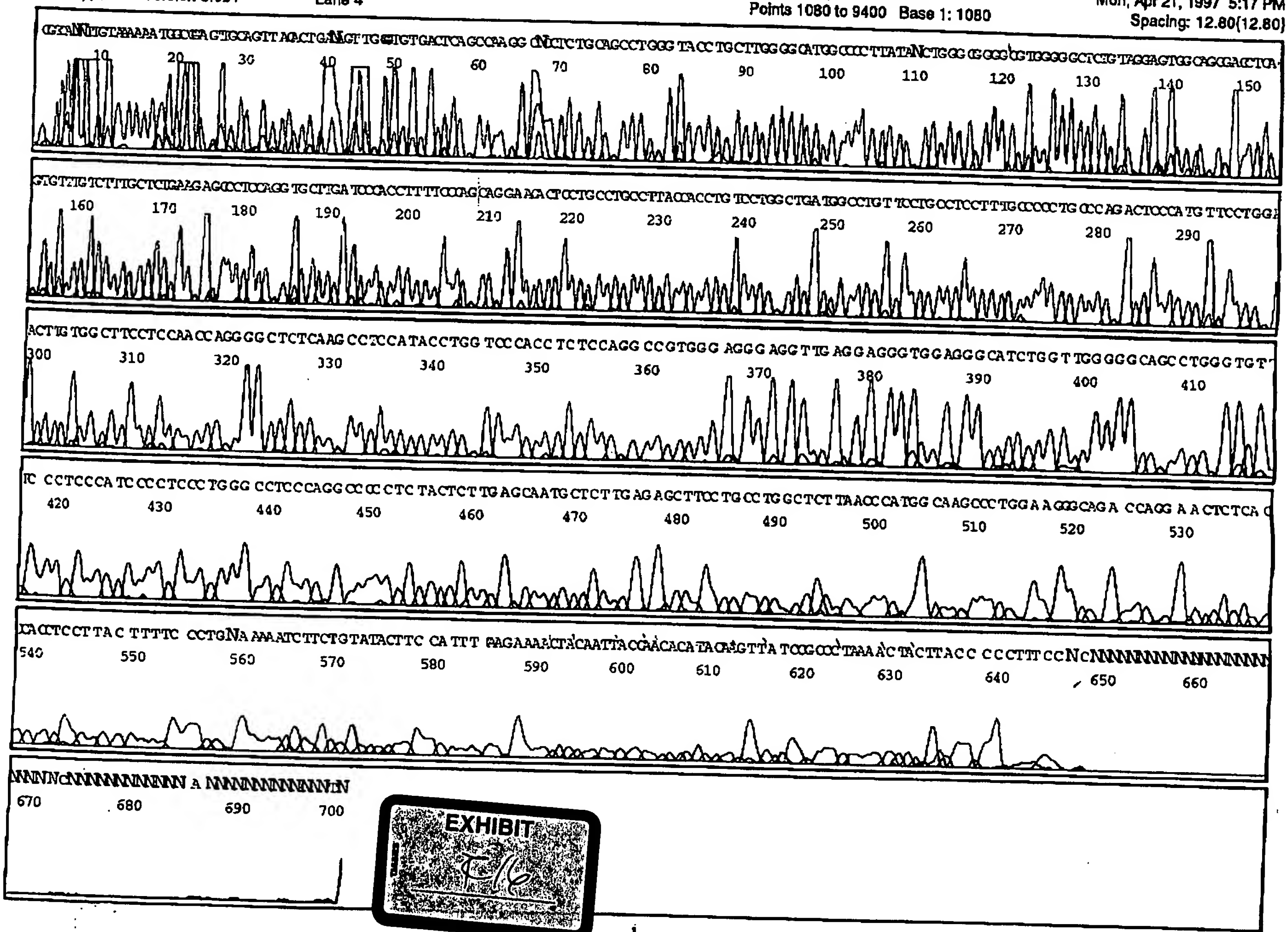
A7-Kostya/wold-17

Kostya/wold-17
Lane 4

p*2,
primer KD10.

Signal G:467 A:559 T:383 C:372
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1080 to 9400 Base 1: 1080

Page 1 of 1
Mon, Apr 21, 1997 7:52 PM
Mon, Apr 21, 1997 5:17 PM
Spacing: 12.80(12.80)



ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

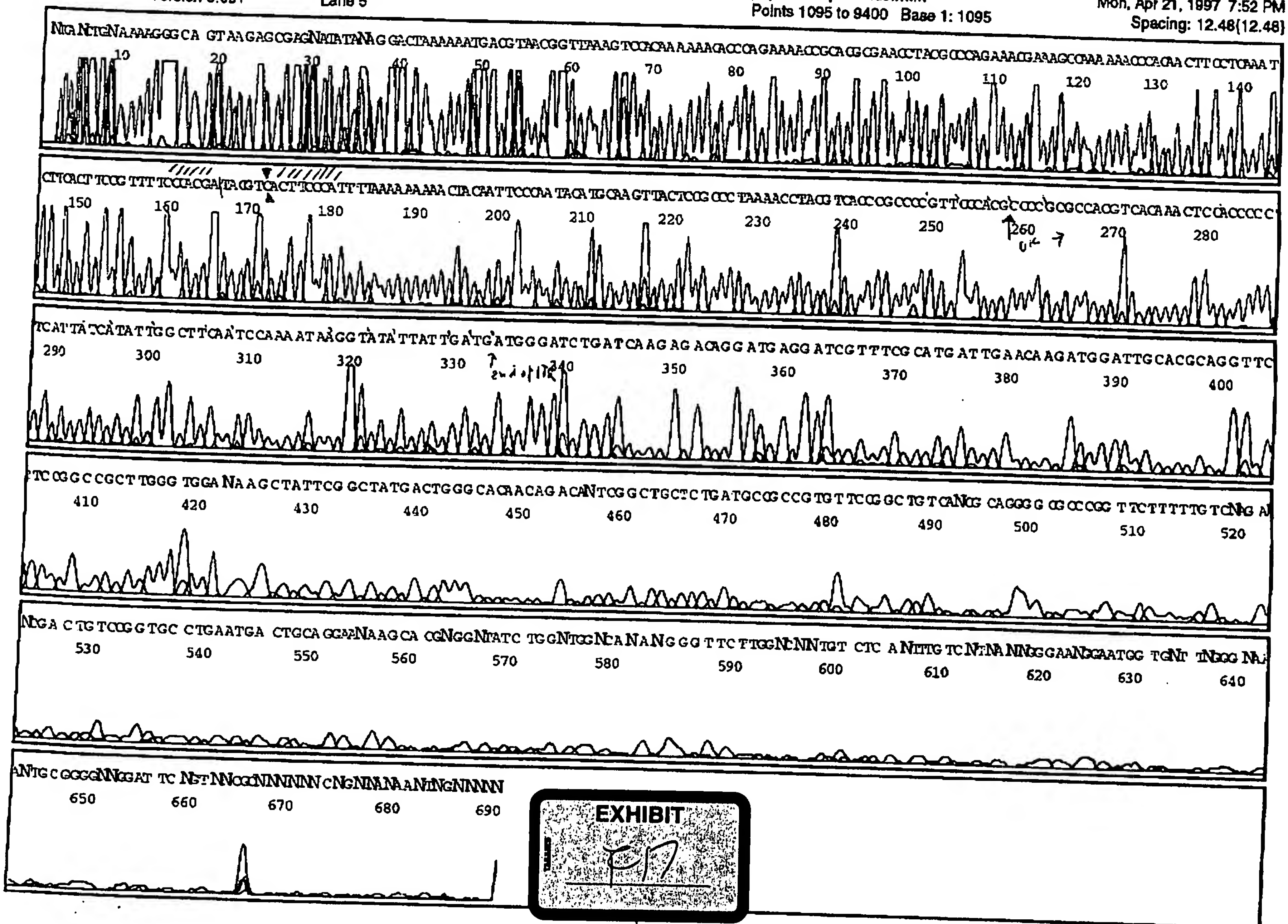
A9-Kostya/wold-18

Kostya/wold-18
Lane 5

p11,
primer wD10

Signal G:256 A:619 T:868 C:512
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1095 to 9400 Base 1: 1095

Page 1 of 1
Mon, Apr 21, 1997 10:27 PM
Mon, Apr 21, 1997 7:52 PM
Spacing: 12.48(12.48)



ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

B4-Wold/KD-#3(27) p142(1), primer(d)/B

Wold/KD-#3(27)
Lane 8

Signal G:221 A:267 T:217 C:217
DT POP6
pop6 dt-seprte-stds.mtrx
Points 795 to 9400 Base 1: 795

Page 1 of 1
Mon, May 12, 1997 10:24 AM
Mon, May 12, 1997 7:49 AM
Spacing: 12.64(12.64)

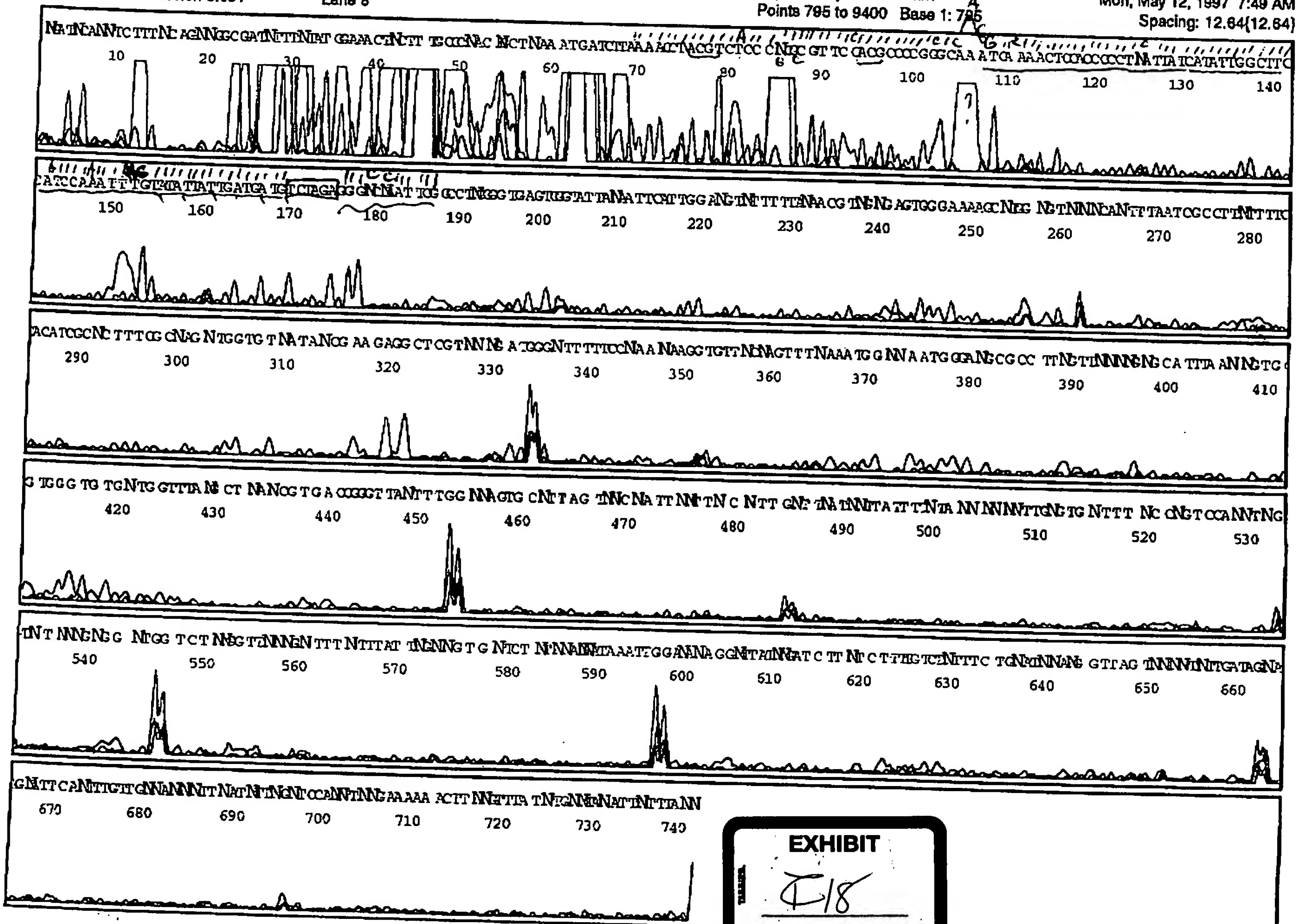


EXHIBIT
E18

p... , primer KD1 +

ABI PRISM

Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1

B6-Wold/KD-#4(28)
Wold/KD-#4(28)
Lane 9

Signal G:373 A:370 T:217 C:189
DT POP6
pop6 dt-seprte-stds.mtrx
Points 1124 to 9400 Base 1: 1124

Page 1 of 1
Mon, May 12, 1997 1:00 PM
Mon, May 12, 1997 10:24 AM
Spacing: 12.19(12.19)

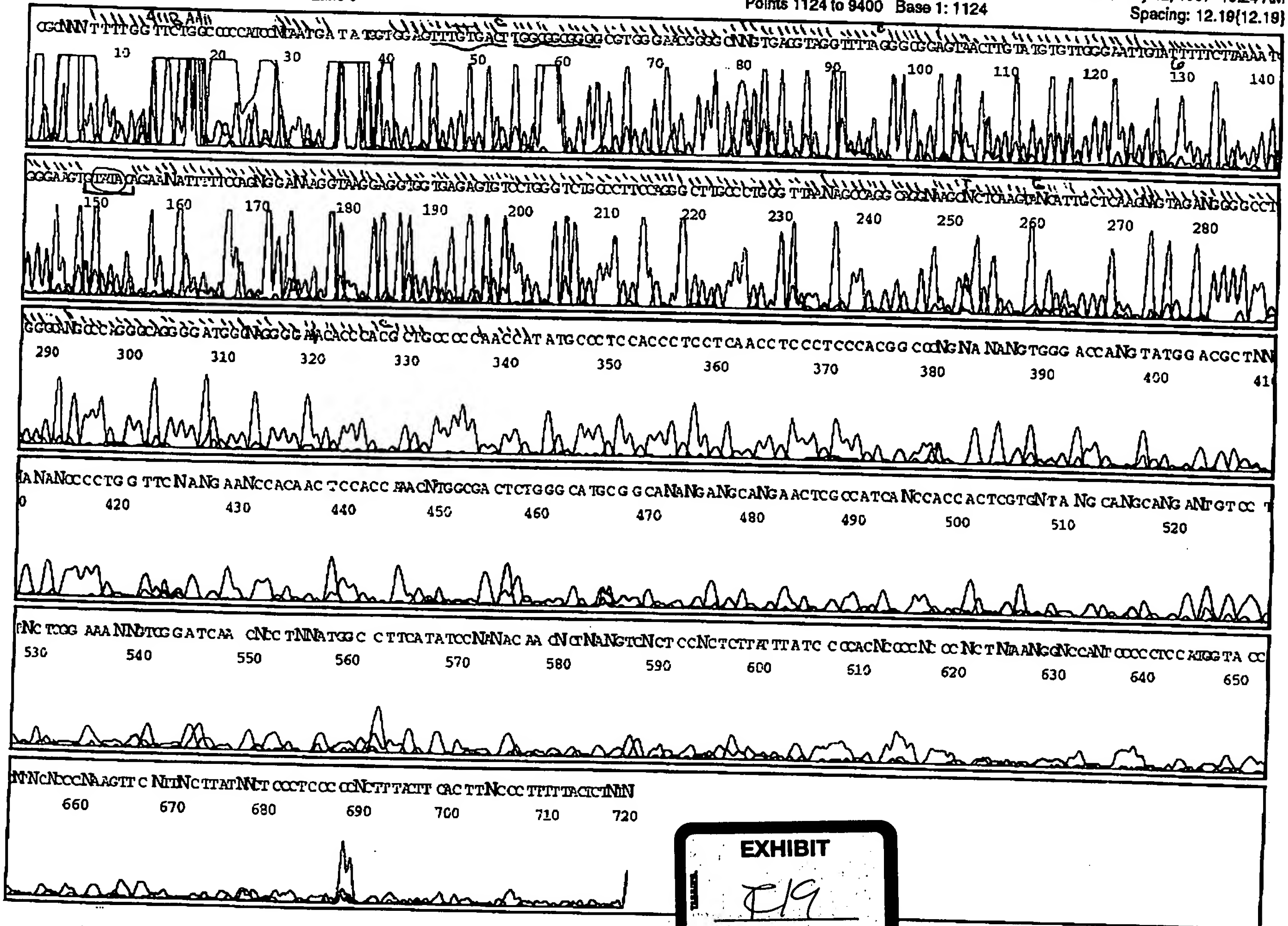


EXHIBIT
E19

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

Doronin #1 PCR product
1101/1107 WD18-19
Doronin #1 Sequence primer WD18 (direct)
Lane 5

Signal G:1955 A:1439 T:1255 C:1906
DT POP6{BD Set-Any Primer}
dRhod2
Points 1100 to 10200 Base 1: 1100

Page1 o
Tue, Mar 23, 1999 10:45 F
Tue, Mar 23, 1999 8:00 P
Spacing: 12.19(12.1

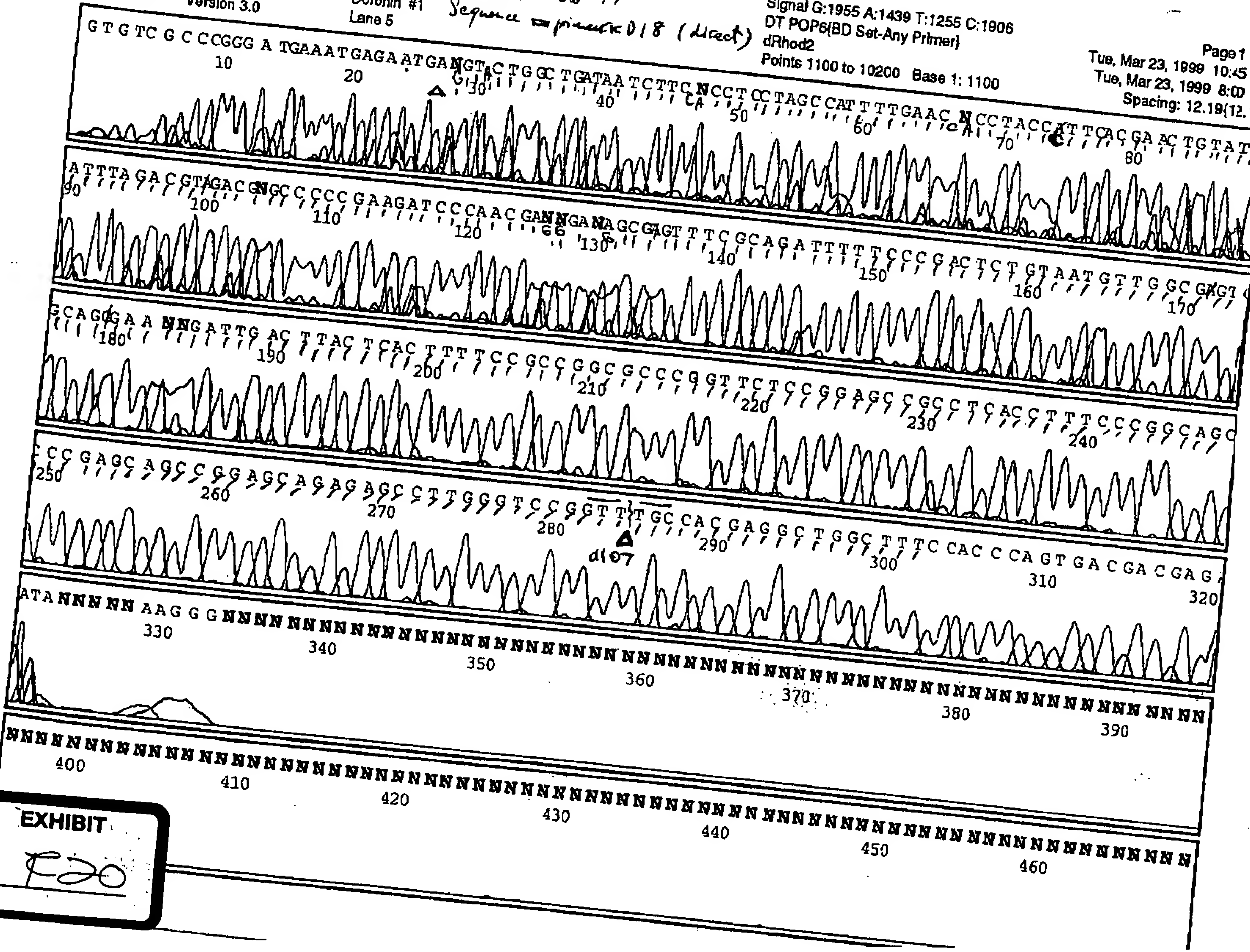


EXHIBIT
F20

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

Doronin #2

Doronin #2
Lane 6

PCR product
1101/1107 WD 18-19
Sequence = primer WD19 (rev)

Signal G:1919 A:1458 T:1194 C:1592
DT POP6{BD Set-Any Primer}
dRhod2
Points 1093 to 10200 Base 1: 1093

Page 1 of 2
Wed, Mar 24, 1999 1:30 AM
Tue, Mar 23, 1999 10:45 PM
Spacing: 12.04(12.04)

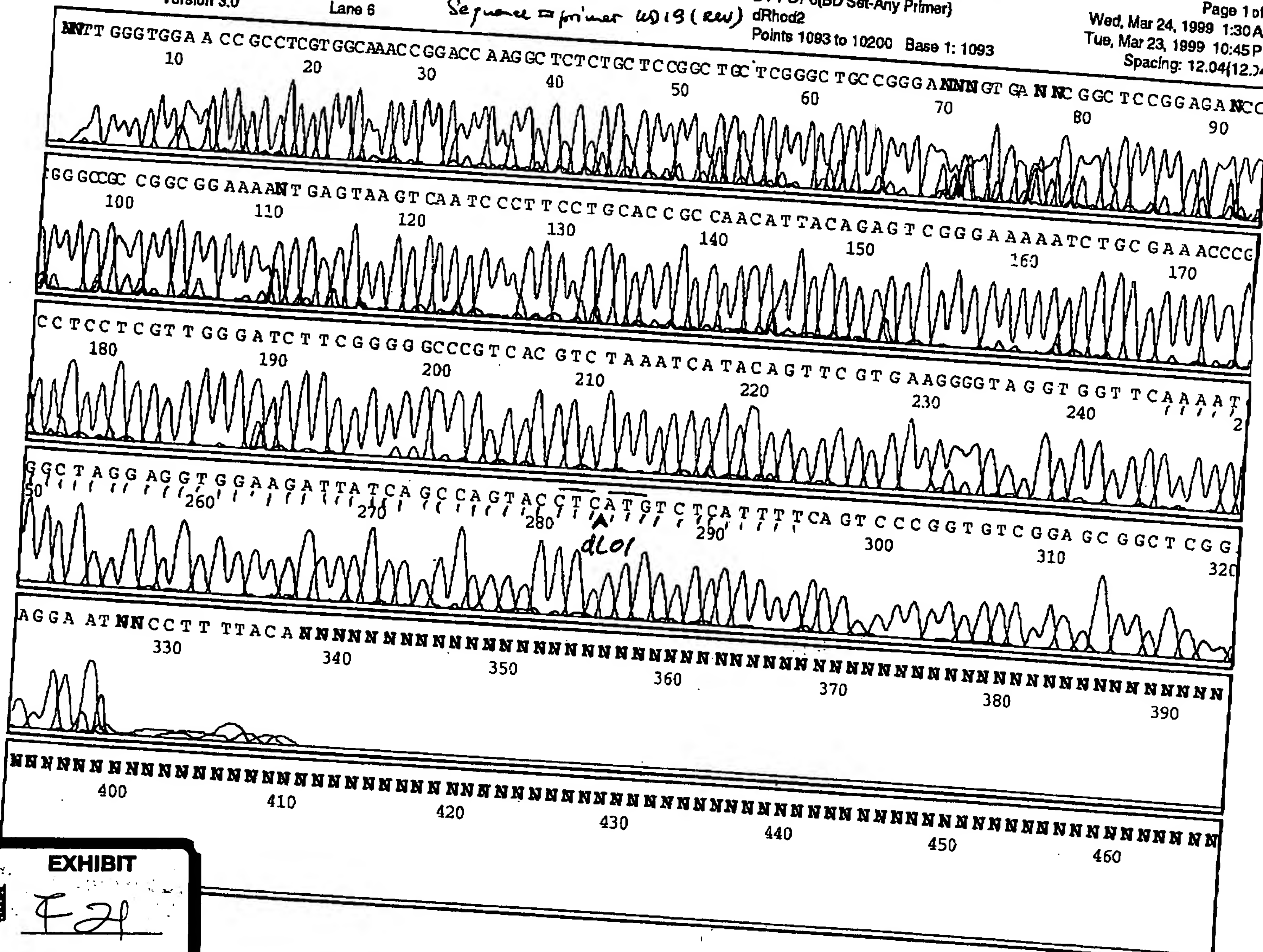


EXHIBIT
F21

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

Doronin #5

Doronin #5
Lane 9

PCR product - u03, u918-19
Sequence => primer u918(Dir)

Signal G:2100 A:1616 T:1484 C:2125
DT POP6{BD Set-Any Primer}
dRhod2
Points 1114 to 10200 Base 1: 1114

Page 1 of 2
Wed, Mar 24, 1999 9:45 AM
Wed, Mar 24, 1999 7:00 AM
Spacing: 12.19{12.19}

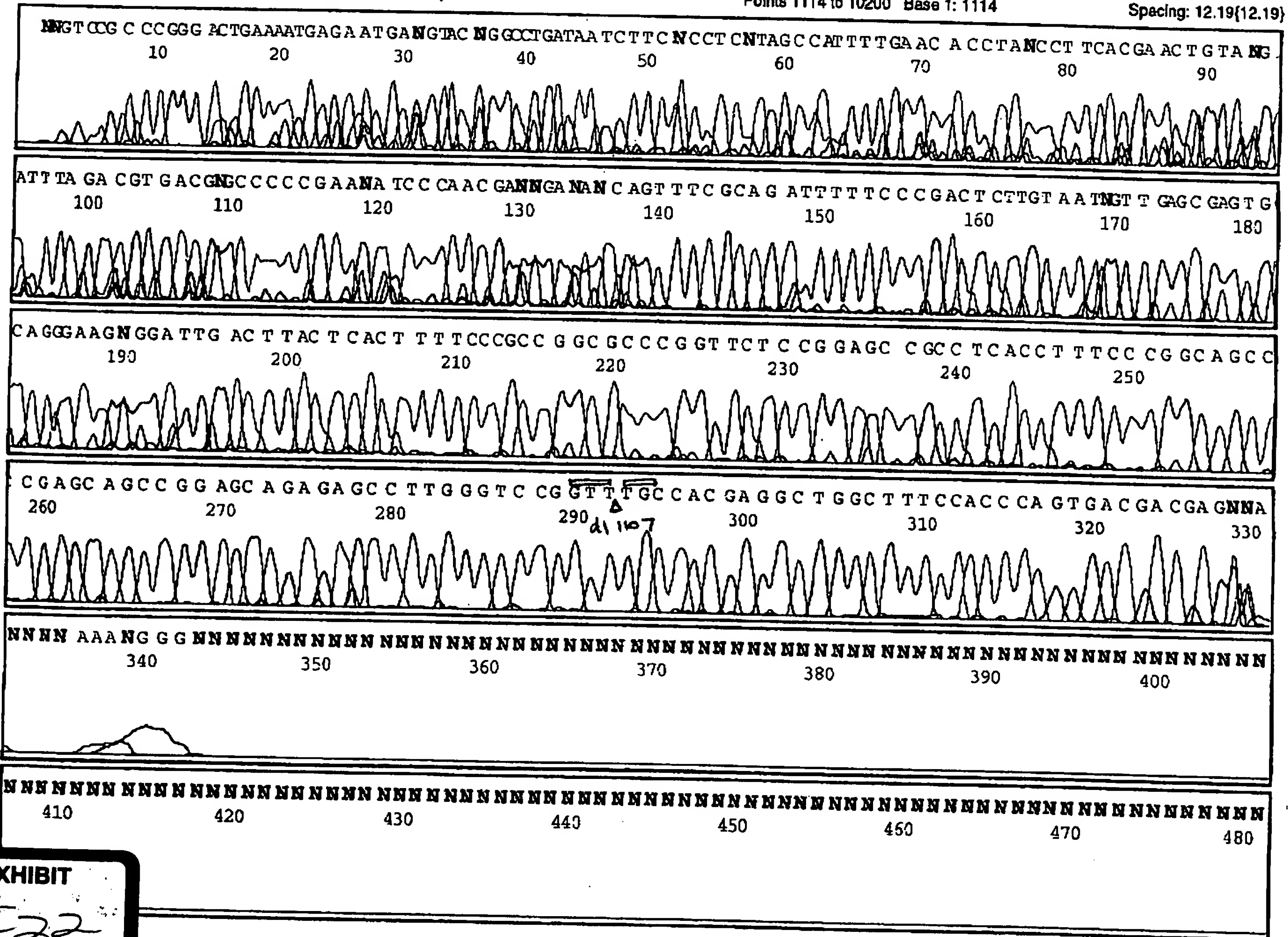


EXHIBIT
E22

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

Doronin #6
Doronin #6
Lane 10

PCR product WD3, WD18-19
Sequence primer WD19 (Rev)

Signal G:2810 A:2515 T:2051 C:2487
DT POP6{BD Set-Any Primer}
dRhod2
Points 1094 to 8440 Base 1: 1094

Page 1 of 2
Wed, Mar 24, 1999 12:09 PM
Wed, Mar 24, 1999 9:45 AM
Spacing: 12.04(12.04)

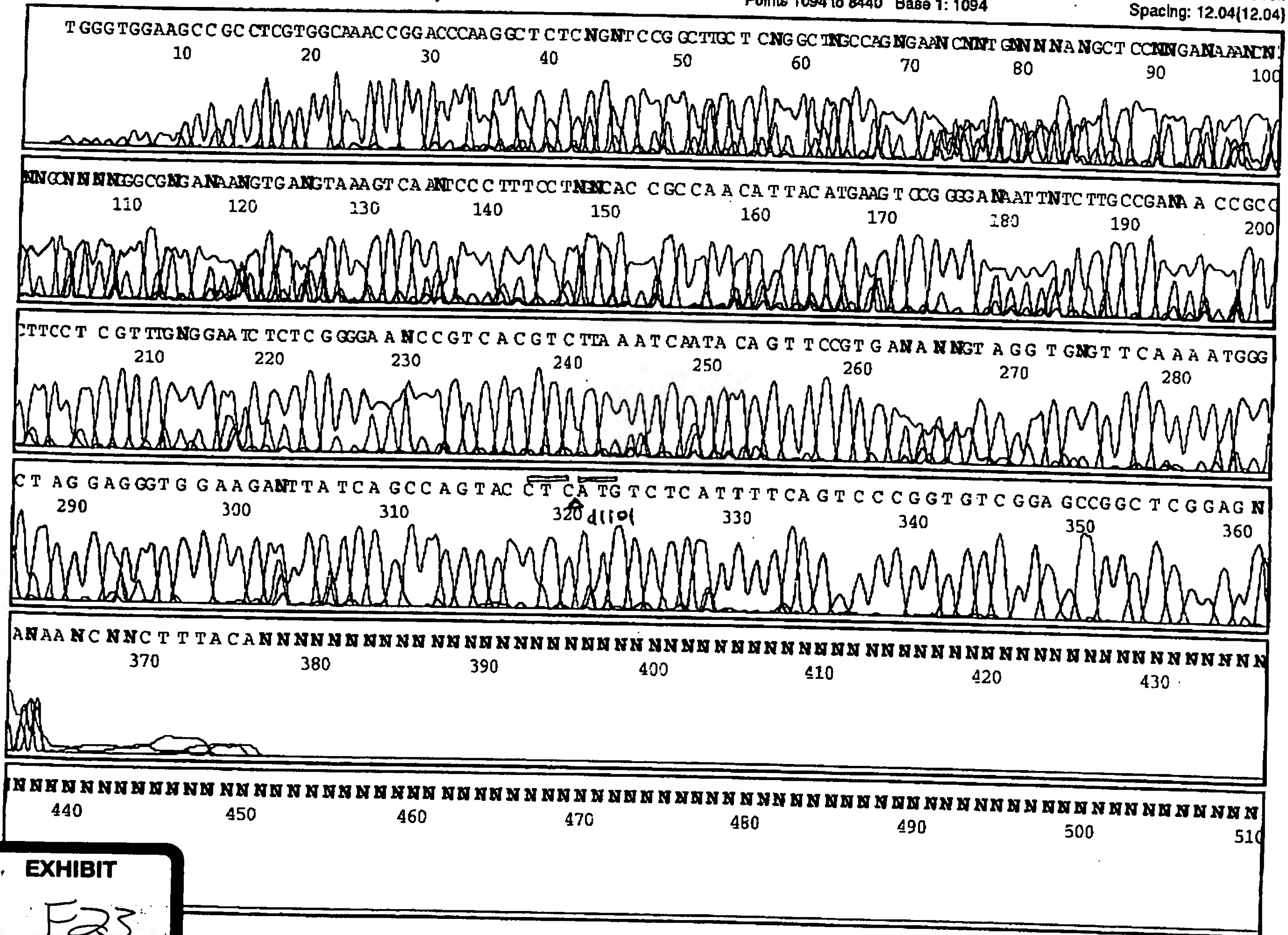


EXHIBIT
F23

EXHIBIT

F24

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

Doronin #7

Doronin #7
Lane 2

pL2 (primer K) 20 (Dir)

Signal G:99 A:69 T:77 C:107
DT POP6{BD Set-Any Primer}
dRhod2
Points 970 to 10200 Base 1: 970

Page 1 of 2
Wed, Mar 24, 1999 5:15 PM
Wed, Mar 24, 1999 2:30 PM
Spacing: 12.04{12.04}

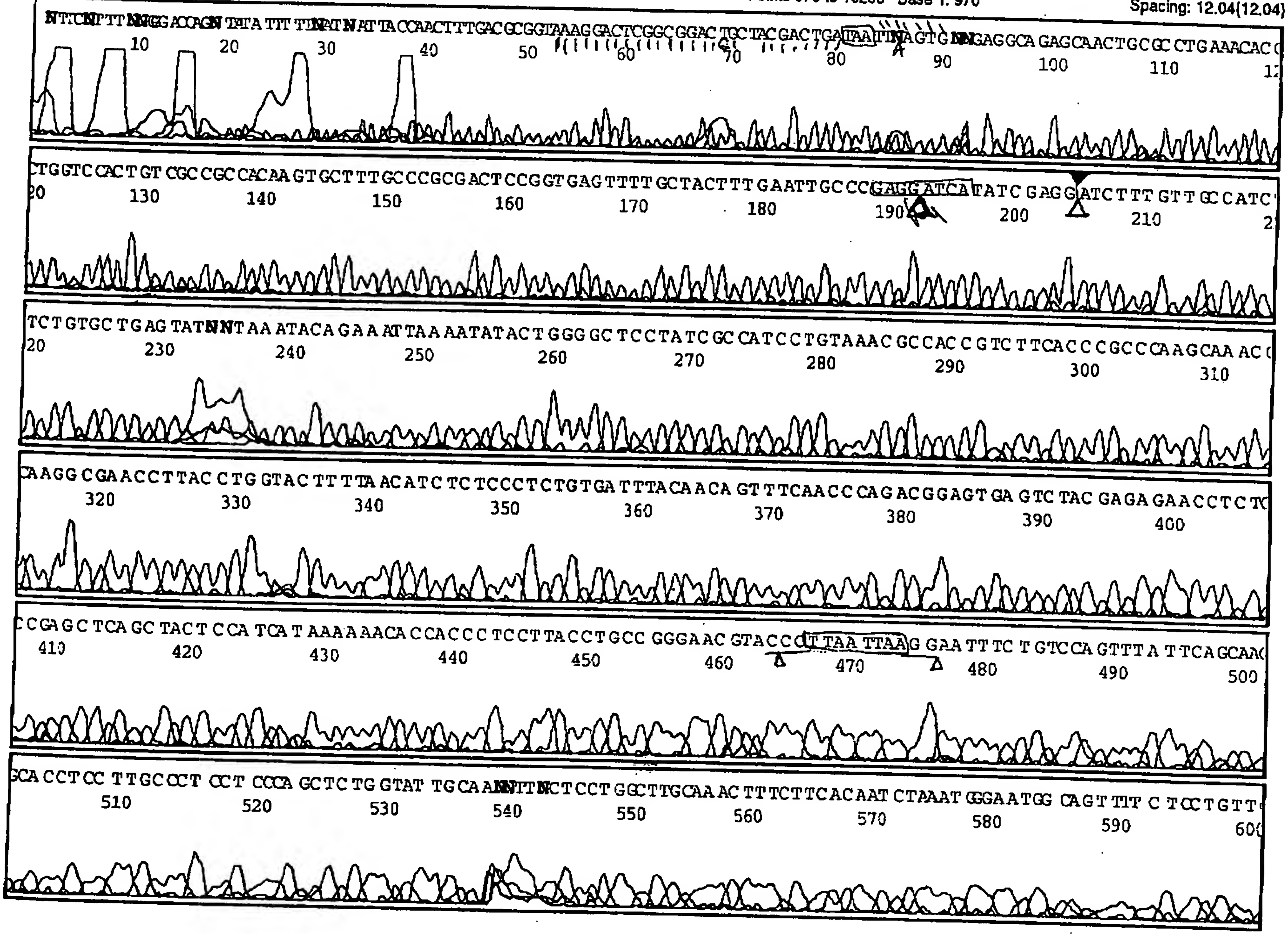


EXHIBIT
F25

ABI PRISM

Model 310
Version 3.0
ABI-CE1
Version 3.0

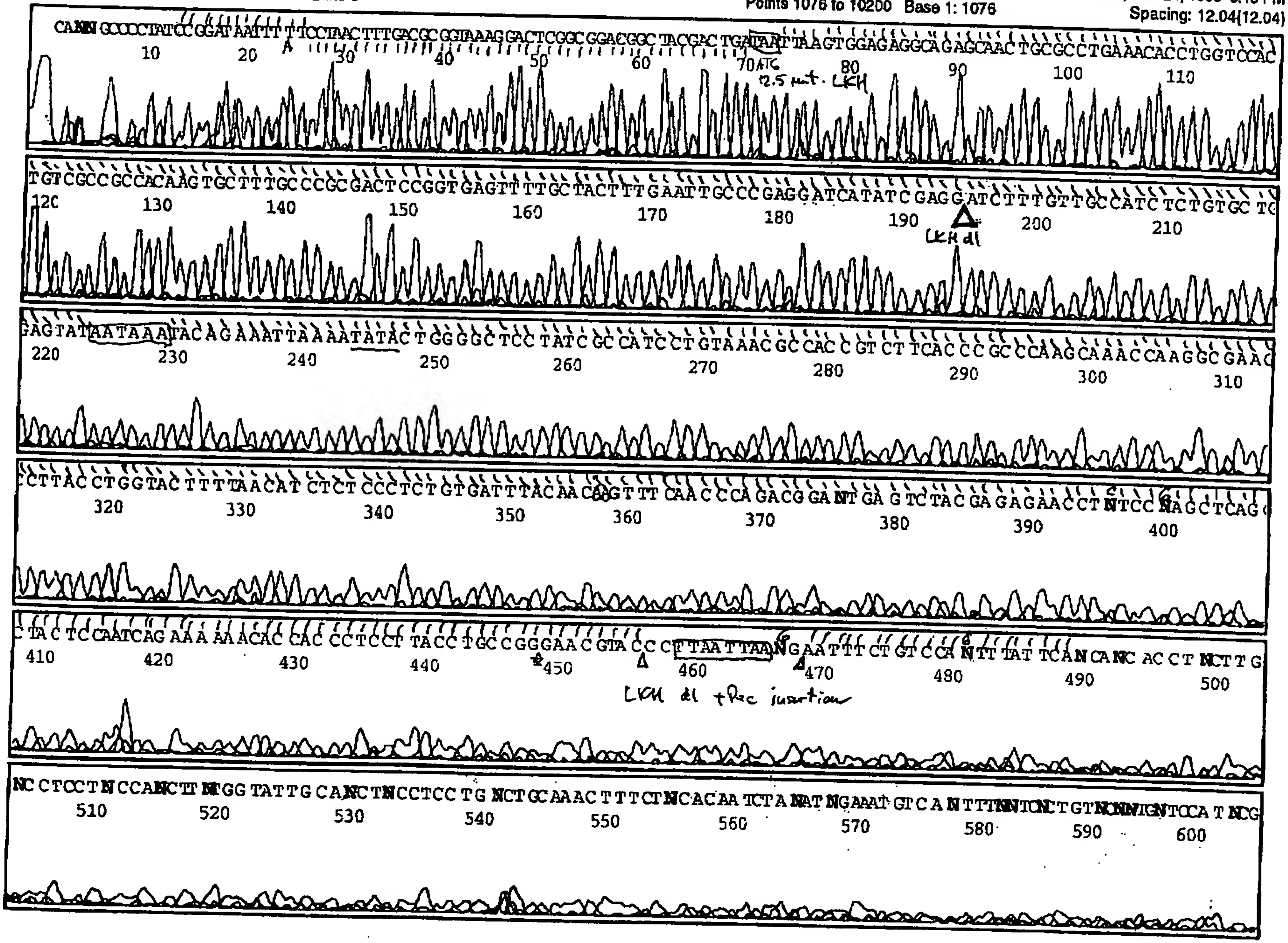
Doronin #8

Doronin #8
Lane 3

pL2, alkaline denaturation
primer 4920 (Dir)

Signal G:65 A:41 T:42 C:53
DT POP6{BD Set-Any Primer}
dRhod2
Points 1076 to 10200 Base 1: 1076

Page 1 of 2
24, 1999 8:01 PM
Wed, Mar 24, 1999 5:15 PM
Spacing: 12.04(12.04)



970619 Infection of A549 (Kostya's Viruses)

A549 (6/18) "1:40" — ~80% confl. (estimate 10^6 cells/TD)

Added SF-DME (2ml), removed & added 1ml SF-DME (~11Am);

Infection at 2:30 pm

Virus	Prep	Titer	Inf'g Volume (1:20 dilution)	# TD
① mock	—	—	—	1
② dl 309 (VS 179) (910308)		1.2×10^{11}	10 µl/TD	4
③ KD-1 (1101/1107 ADP)	(970508)	9.64×10^{10}	15 µl/TD	4
④ KD-2	(970611)	estim $4\text{-}5 \times 10^{10}$	20 µl/TD	4
⑤ KD-3	970617; ~15ml 32ag Sup) 150cm² flask	estimated $2\text{-}3 \times 10^{10}$	25 µl/TD	3
			40 µl/TD	1 (Ara-C TD)

Added 1½ ml DME (10% FCS) at 3:30pm.

Added 4X Ara-C (10µl of stock) at ~6 pm & again at 9 Am on (6/20), fixed Ara-C & one set of coverslips at 27h post-infn. (other cs's fixed at 49 hours post-infection → all fixed in Paraformaldehyde followed by MeOH/DAPI, then a 2 min MeOH rinse & rehydrated in PBS; stained on (6/23) [after checked out Baoling's antibodies]

EXHIBIT

44

970623 Immunofluorescence Staining

all fixed in PF/MeOH ō DAPI

Set #1 (M73(1:2) 121531 (1:100 final)) 600 μl

1 mock (Ara-C) slight bckgrd (all, Ab)
2 dl 309 (Ara-C) ~100% inf'd; some Golgi
not n.e. 3 dl 309 (27h) Golgi + speckled around nuclei
4 KD-1 (Ara-C) ~80-90% inf'd; some Golgi
5 KD-1 (27h) much more than Ad5; very vesicular
6 ↓ (49h) all cells bright (all vesicular)
7 KD-2 (Ara-C) 70-80% Inf'd; prob. some Golgi
8 ↓ (27h) more like Ad5, but some nuclei (intensely stained)
9 ↓ (49h) more diffuse; some vesicles; some cells quite bright; & Golgi
10 KD-3 (Ara-C) 50-60% inf'd; pretty similar to Ad5 (AC)
11 ↓ (27h) not too much staining
12 ↓ (49h) some cells brighter (again rather "diffuse")

Set #2 (αDBP/αFiber each 1:400) 250 μl

13 mock (49h) slight backgrd (esp. FITC)
14 309 (27h) all nuclei stain for both
15 KD-1 (49h) strong staining for both
16 KD-2 ↓ more stained for DBP
17 KD-3 ↓ fewer cells stained for Fib

A-549 (6/18) 1:40 (p100) – Inf'd (6/19)
volume of 1: dil: 309 (10 μl), KD-1 (15 μl), KD-2 (20 μl), KD-3 (40 μl (Ara-C), or 25 μl)

1st Ab(s): 50 μl for 60 minutes;

PBS rinses: 70 min, 12 min;

2nd Ab(s): Goat αRabbit FITC (970612) + Goat α Mouse-RITC (not preadsorbed)
1:50 dilution of each
50 μl for 34 minutes;

PBS rinses: 6½ min, 5 min;
dd H_2O dip
mounted in Elvanol ō p-phenylenediamine

EXHIBIT #2

970 (5/20) Plaque Assays (New Counts)

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
① dl751	.5*10^-8	A	76	74	TMTC →				
		B	65	66	TMTC →				
	.5*10^-9	A	10	12	10	9	0	0	0
		B	5	6	bact.	5	0	0	TMTC
		C	4	6	10	10	2	0	2
	.5*10^-10	A	2	0	2	3	0	0	0
		B	0	0	0	0	0	0	0
		C	1	0	1	0	0	0	0
② dl707 (4/23/97)	.5*10^-8	A	10	37	15	30	31	10	TMTC
		B	6	37	15	17	26	11	TMTC
	.5*10^-9	A	3	3	2	2	2	5	0
		B	0	6	0	3	5	3 (bact)	bact
		C	0	2	0	0	12	9	2
	.5*10^-10	A	0	0	0	0	1	0	0
		B	0	0	0	0	0	0	0
		C	0	0	3	0	1	0	0
③ dl707 (3/15/97)	.5*10^-8	A	16	41	14	68	TMTC	19 →	
		B	44	51	15	47	TMTC	28 →	
	.5*10^-9	A	2	2	2	8	13	3	0
		B	1	8	5	2	8	5	2
		C	0	2	2	9	14	6	2
	.5*10^-10	A	0	0	0	0	1	0	0
		B	0	0	0	0	0	1	0
		C	0	0	0	0	0	3	0

EXHIBIT
#4

970520 Plaque Assays

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
		[illegible]	42	80					
4 PME	.5*10^-8	A	[illegible]	[illegible]	74	TMTC →			
		B	37	102	78	TMTC →			
	.5*10^-9	A	1[illegible]	8[illegible]	16	12	4	0	TMTC
		B	7	17	11	14	1	0	TMTC
		C	7	15	20	7	11	0	TMTC
	.5*10^-10	A	0	0	4	1	2	1[illegible]	2
		B	0	0	3	0	1	0	0
		C	0	2	2	2	0	2	0
5 KD	.5*10^-8	A	TMTC →						
		B	TMTC →						
	.5*10^-9	A	20	8	8	3	5	0	0
		B	37	7	2	1	2	0	0
		C	24	11	11	1	0	0	0
	.5*10^-10	A	2	0	1	1	1	2	0
		B	0	1	0	0	0	1	0
		C	5	0	0	2	1	2	0

EXHIBIT

#5

Cumulative Counts

970 (5/20) Plaque Assays

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
(1) dl751	.5*10^-8	A	76	150	TMTC →				
		B	65	131	TMTC →				
	.5*10^-9	A	10	22	32	41	41	41	41
		B	5	11	11	16	16	16	16
		C	4	10	20	30	32	32	34
	.5*10^-10	A	2	2	4	7	7	7	7
		B	0	0	0	0	0	0	0
		C	1	1	2	2	2	2	2
			22	46	69	96	98	98	100
(2) dl707 (4/23/97)	.5*10^-8	A	10	47	60	90	121	131	TMTC
		B	6	43	58	75	101	112	TMTC
	.5*10^-9	A	3	6	9	11	13	18	18
		B	0	6	6	9	14	17	17
		C	0	2	2	2	14	23	25
	.5*10^-10	A	0	0	0	0	1	1	1
		B	0	0	0	0	0	0	0
		C	0	0	3	3	4	4	4
			3	14	20	25	46	63	65
(3) dl707 (3/15/97)	.5*10^-8	A	16	57	71	139	TMTC →		
		B	44	95	110	157	TMTC →		
	.5*10^-9	A	2	4	6	14	27	30	30
		B	1	9	14	16	24	29	31
		C	0	2	4	13	27	33	35
	.5*10^-10	A	0	0	0	0	1	1	1
		B	0	0	0	0	0	1	1
		C	0	0	0	0	0	3	3
			3	15	24	43	79	97	101

EXHIBIT

#6

970520 Plaque Assays

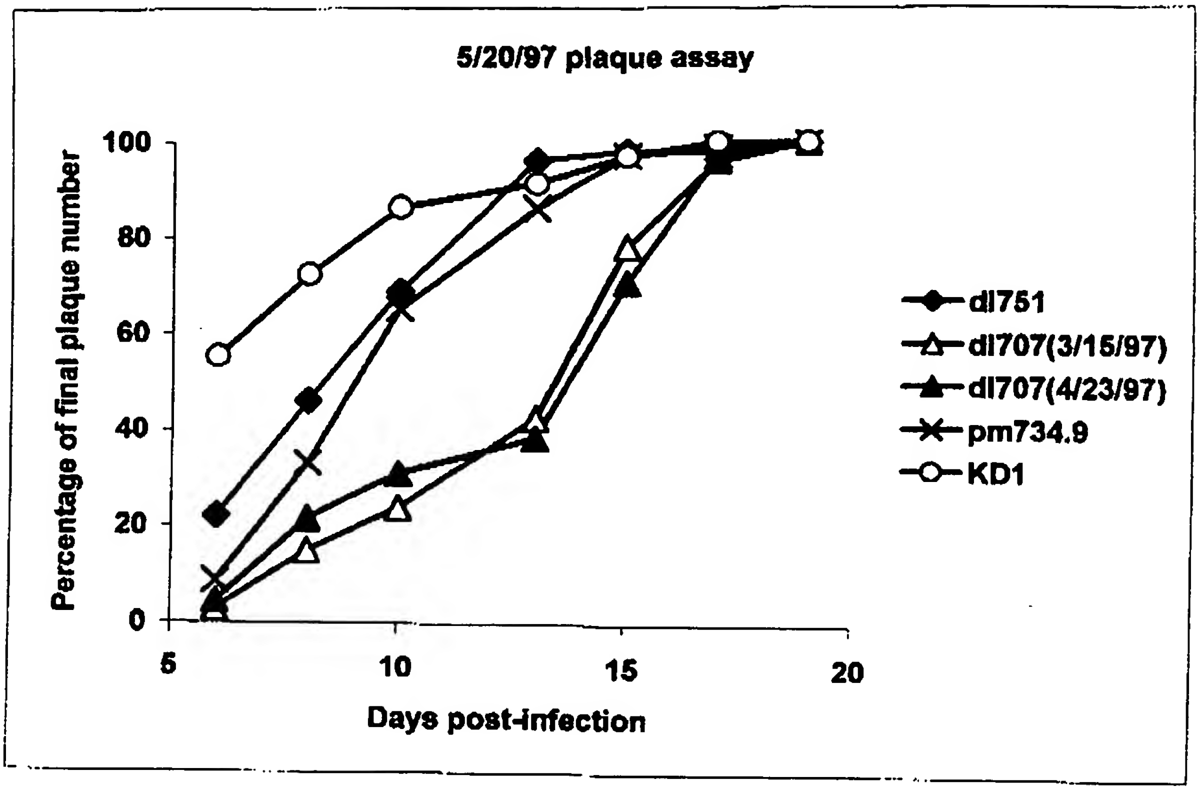
Virus	Dilution	Dish #	5/26	5/28	5/30	6/2	6/4	6/6	6/8
4 PME	.5*10^{-8}	A	42	122	196	TMTC	→		
		B	37	139	217	TMTC	→		
	.5*10^{-9}	A	1	9	25	37	41	41	TMTC
		B	7	24	35	49	50	50	TMTC
		C	7	22	42	49	60	60	TMTC
	.5*10^{-10}	A	0	0	4	5	7	8	10
		B	0	0	3	3	4	4	4
		C	0	2	4	6	6	8	8
			15	57	113	149	168	171	173
5 KD	.5*10^{-8}	A	TMTC	→					
		B	TMTC	→					
	.5*10^{-9}	A	20	28	36	39	44	44	44
		B	37	44	46	47	49	49	49
		C	24	35	46	47	47	47	47
	.5*10^{-10}	A	2	2	3	4	5	7	7
		B	0	1	1	1	1	2	2
		C	5	5	5	7	8	10	10
			88	115	137	145	154	159	159

EXHIBIT

#7

970520 plaque assay*

	6	8	10	13	15	17	19
dl751	22	46	69	96	98	98	100
dl707(3/15/97)	3	14.9	23.8	42.6	78.2	96	100
dl707(4/23/97)	4.6	21.5	30.8	38.5	70.8	96.9	100
pm734.9	8.7	32.9	65.3	86.1	97.1	98.8	100
KD1	55.3	72.3	86.2	91.2	96.9	100	100

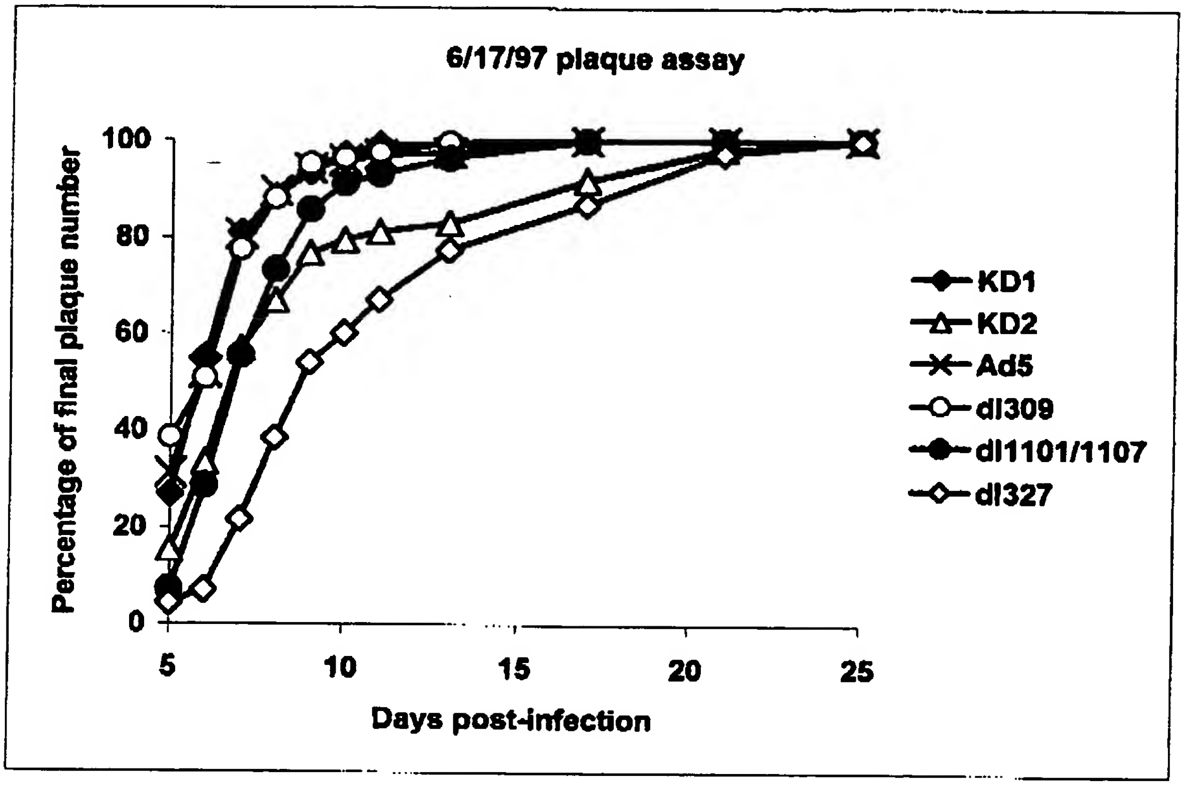


*numbers at the top of the page are the data points represented in the graph

EXHIBIT
48

970520 plaqu assay*

	5	6	7	8	9	10	11	13	17	21	25
KD1	27	55.2	80.9	88.4	93.4	97.1	99.2	99.2	100	100	100
KD2	15.3	33.3	56.8	66.7	76.6	79.3	81.1	82.9	91.9	98.2	100
Ad5	31.2	51.9	80.6	89.5	94.1	95.8	97	97.5	100	100	100
dl309	38.5	50.8	77.5	88.1	95.1	96.3	97.5	99.6	100	100	100
dl1101/1107	7.1	28.6	55.4	73.2	85.7	91.1	92.9	96.4	100	100	100
dl327	4.3	7	21.7	38.3	53.9	60	67	77.4	87	97.4	100



*numbers at the top of the page are the data points represented in the graph

EXHIBIT
49

(7/26) Blue (5/28) Green

H6

970526 (5/20) Plaque Assays

	Virus	Dilution	A	B	C	Comments
1	dl751	$0.5 * 10^{-8}$	76 +74	65 +66	~~X~~	quite large + very distinct
		$.5 * 10^{-9}$	10 +12	5 +6 (bact.)	4 +6	
		$.5 * 10^{-10}$	2 +0	0 0	1 +0	
2	dl 707 (4/23)	$.5 * 10^{-8}$	10 +37	6 +37	~~X~~	small, flat + very indistinct
		$.5 * 10^{-9}$	3 +3	0 +6	0 +2	
		$.5 * 10^{-10}$	0 0	0 0	0 0	
3	dl 707 (3/15)	$.5 * 10^{-8}$	16 +41	44 +51	~~X~~	flat + indistinct
		$.5 * 10^{-9}$	2 +2	1 +8	0 +2	
		$.5 * 10^{-10}$	0 0	0 0	0 0	
4	PME	$.5 * 10^{-8}$	42 +80	37 +102	~~X~~	fairly large + quite distinct
		$.5 * 10^{-9}$	1 +8	7 +17	7 +15	
		$.5 * 10^{-10}$	0 0	0 0	0 +2	
5	KP	$.5 * 10^{-8}$	TMTC	TMTC	~~X~~	small to large + all distinct; "cleared" center in many plaques (all cells lysed?)
		$.5 * 10^{-9}$	20 +8	37 +7	24 +11	
		$.5 * 10^{-10}$	2 +0	0 +1	5 +0	
6	mock	—	0	0	~~X~~	

AT Counts + descriptions of Shari O'Briens (5/20) Plaque Assay

Comments most likely done on (5/21)

EXHIBIT

#10

Analysing the infectivity of the viruses 1101/1107 and dl309 on Growth arrested and growing cells.

cell line = HEL 299 human Lung embryonic.

Grown as in ATCC literature

T. 75 flask 1 from Karl,

split to 1:30 = 35 mm dish.
1:60 = 35 mm dish. (how many)
(for growing condition)

35mm dish. uninfected 1101/1107 dl309

1) 35mm + cover glass

Growth arrested / Growing

how many days? ~~2~~ ~~2~~ 6 6 6 6 ~~28~~
6 days

2) 35mm + Coverslip =

how many days? 6 days

titer each point 1-6 days or

EXHIBIT
I1

Analysis of the dl 1101/1107, dl309 viruses infectivity on Growth arrested and Growing HEK 293 cells:

Hek 293 one T75

35 mm dishes 1:30 split for growth arrested
1:60 for growing cells

		Growing	Growth arrested
	1. Uninfected-mock	1 ~~+2~~	1+2
MOI: 100	2. 1101/1107 infected	2	2
"	3. dl 309 infected	2	2

look for CPE - days?

4. On Cover slip # 1, 2, 3 ~~2 2 2~~ 1+6 days → for Ad fiber IF.

12

10

22

virus location
dl309 = pfu: ✓
dl1101/1107 = pfu: ✓

1.

EXHIBIT
I 2

HEL 299

↓ T-75

35 mm dishes 1:30, 1:60

↓

MEM ; 0.1mM Non essential a.as ; 1mM Sodium-pyruvate + 0.1 % lactalbumin hydrolysate.
+ 10 % FBS

1:60 split, count the cells + infect c̄ dl 1101/1107, dl 309. 100 moi

1:30 split, allow to become confluent, then growth arrest by low serum (0.2 %) (3 days) then infect c̄ dl 1101/dl 1107, dl 309

↓

EXHIBIT
#3

viral infection dl 1101/1107

- Count some growing HEL 299 cells.
- 100 pfu -

- Make 200 ml EMEM + Na Pyruvate, NEAA + Lactalbumin hydrolys
100x 100x 100x

$$\frac{116 \quad 31}{547 \times 2} = 294 \quad \boxed{294{,}000 / ml}$$

Location

dl 309 - 950111 - pfu 2.15×10^{11} 5-7

dl1101/1107 - from Karl - pfu 1.95×10^{11}

for 35 mm - HEL 299 growing cells - counted - 294,000 / ~~total~~ ml

100pfu dl 309 $= \frac{\cancel{100} \times 294\cancel{,000}}{2.15 \times 10^{\cancel{9}\,6}}$

$= \frac{294}{2.15 \times 10^{\cancel{6}\,4}} = \frac{2.94}{2.15 \times 10^4} = 0.00013$

= virus is too concentrated.
Dilute to 10^9

10 μl dilute to 1 ml in medium (~~No~~ serum) gives
- 2.15×10^9

$= \frac{\cancel{100} \times 294\cancel{00}}{2.15 \times 10^{\cancel{7}\,4}} = \frac{294}{2.15 \times 10^4}$

dl 1101/dl1107 100 pfu = $\frac{\cancel{100} \times 294,\cancel{000}}{1.95 \times 10^{4}}$ = $\frac{294}{1.95 \times 10^4}$

dilute the virus to 10^9
i 10 μl to 1 ml

= $\boxed{15\ \mu l}$

12/16/96

Growing cells

2 × 35mm dish dl 309, 100 pfu

2 × 35mm dish dl 1101/1107, 100 pfu

6 × 35mm on coverslip dl 1101/1107 100 pfu

EXHIBIT
15

01

Growth arrested HEL 299 : infection c̄ dl309, dl1101/1107

35 mm. dish Medium + 0.2% FCS – 3 days

Cell counts – 341,000

dl 308 = 100 pfu = $\frac{341 \times 10^{3} \times 100}{2.15 \times 10^{9}}$ = 15.8 /ml

dl 1101 = 100 pfu = $\frac{341 \times 10^{3} \times 100}{2.45 \times 10^{9}}$ = 17.4 ul

Virus 10 ul to 1ml → 10^{9}

↓

37°C / 1hr

↓

Medium + 0.2% FCS.

Fixing the HEL299 cells c̄ Methanol + DAPI (1mg/ml)
(1µl to 1ml methanol)

1. Wash the cells 2x PBS w/o
2. Fix c̄ Methanol (-20°) + DAPI ~ 8 min (-20°C)
3. Wash 1x Methanol w/o DAPI
4. Wash c̄ 1x PBS, store at 4°C

✓	1 day	12/17		
✓	2 day	12/18		
✓	3 day	12/19/96	→	c̄ dl309 started showing CPE
✓	4 day	12/20	— NO CPE	
	5 "	12/21	— NO CPE	— dl309 complete CPE
	6 "			
	7 day	12/23	— NO CPE	all dl309 cells infected
	8 day	12/24	— NO CPE	rounded & came off
	9	12/25		
→	10 day	12/26	— CPE?!	cells looks different in dl 1101/1107
F	11	12/27		
S	12	12/28		— CPE? starts on 12th day dl 1101/1107
Sun	13	12/29		
✓ M	14	12/30	— CPE?	spreads - dl 1101/1107
	15	12/31	— "	"
	16	1/97		
✓	17	2/97	→	Take Photographs.
	18	3/	→	
	19	4/	→	
	20	5/	→	almost all the cells died - dl 1101
	25th	10/	→	Stain dish, c̄ crystal violet 2 dl 1101/1107 2 - dl309

EXHIBIT
21

Growth arrested HEL 299 cells:-

Date	Days	
8	15	NO visible CPE in dl 1101 / 1107 : Some cells are floating. In normal HEL 299 cells Some cells are floating but less.
9	16	same as above.
10	17	"
11	18	same
12	19	cells looking different than norm ~~al~~ HEL 299. But, dl 309 CPE looks differe
13	20	
14	21	"
15	22	"
16	23	" CPE?!
17	24	" CPE?!
18		

EXHIBIT
I8

HEL299 cells

17 Days

7

Kodak Tmax 100
~~black & white~~ print

Growing HEL 299:

TMAX

1.	mock	N
2	"	+1
3	"	−1
4	"	−1
5	"	N
6	"	+1
7	d1 1101/1107	N
8	"	+1
9	"	−1
10	"	N
11	"	+1
12	"	−1
13	"	N
14	"	+1
15	"	−1
16	"	N
17	"	+1
18	"	−1
19	"	N
20	"	+1
21	"	−1
22	"	N
23	"	+1
24	"	−1
25	"	N
26	"	+1
27	"	−1
28	"	N
29	"	+1
30	"	−1
~~31~~	~~"~~	
32	"	N
33	"	+1
34	"	−1
~~35~~	~~mock~~	~~N~~
~~36~~	~~mock~~	~~+1~~

EXHIBIT

29

IF: HEL 299 : Growing:

1. ab - Fiber, → 1:400) 30'
DBP → 1:400

2. Goat anti mouse Rhodamine 1:50 (not preabsorbed
Goat anti Rabbit FITC 1:25..

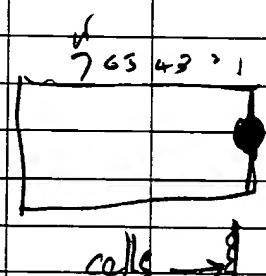
FITC = DBP
RITC = Fiber

1 = 1st day
2 = 2nd
3 = 3rd
4 = 4th
5 = 14th
6 = 17th
7 = CNTRL

d1101/1107
- ? 4
- little broken

α DBP p67
66420
850625
α Ad2 Fiber 4D2-5
ascites fluid

1. ab = Ad Fiber dil 1:400) in 1 tube.
= DBP dil 1:400.
35 μl / disk → 37c / 30 min
↓ wash: c̄ 1xPBS w/o, 10 min x 3 times.



1 2 3

2. Goat α mouse Rhodamine = 1:50) 960/25
Goat α Rabbit FITC = 1:25
37c / 15 min

8 → 400
16 → 400
1,2,

376
8
16
400

Growth Assay

Growth arrested cells.

HEL 299

T-75 → split 15 x 35mm dish.

dishes same like Growing

↓

add EMEM + 0.2% FCS

↓

3 days

↓

infect c̄ dl 1101/ 1107, pM 975

the control dl 309.

total dish # 27

Growth Assay

MEL 299 T-75 Growing:

split to 35 x 35mm dish

(1) 1 + 10 dish days (1, 4, 6, 8, 10, 12, 14, 16, 18, 20)
dl 1101/1107

(2) 1 + 10 dish Same days - pm 975

infect 100 pfu, freeze the dishes as days shown.

(3) 4 dishes infect c̄ dl 309 for full infection

Freeze 1, 4, 6 days

total dishes 27

EXHIBIT

1/14/98

Infection dl 1101/1107 pfu 1.95×10^{11}
dl 309 2.15×10^{11}
pm 975 4.06×10^{10}

Growing MEL 299 cells counted: 212,000/ml

(1) 100 pfu

For dl 1101/1107 $= \frac{\cancel{100} \times 212000}{1.95 \times 10^{\cancel{9}} 2} = \frac{2.12}{1.95 \times 10^2} = 10.8$ (above: 2.12×10^5)

Dilute the virus to 10^9
10 ml to 1ml medium No serum

(2) For pm 975 $= \frac{\cancel{100} \times 2.12 \times 10^5}{4.06 \times 10^{\cancel{9}} 2} = \frac{2.12}{4.06 \times 10^2} = 5.2$ ml

dilute the virus to 10^9
50 ml to 500 ml serum free medium =

Growth Curve

Growing cells: Summary

(3) the CNTRL

virus dl1309 $= \frac{10 \times 2.12 \times 10^8}{2.15 \times 10^4 \times 2}$ $= \frac{2.12}{2.15 \times 10^2} = 9.8 \mu l$

dilute the virus to 10^9

10 μl to 1 ml serum free media

1. wash the cells c̄ PBS w/o.
2. infect in serum free media 37c / 1 hr
3. After 1 hr, remove the media, wash c̄ 1x media
4. Add EMEM + 10 % FCS.

Freeze the cells after

	1 day	G1	1/15/97 ✓
1-17-97	3 day	G2	dl1309, pm975 shows CPE NO CPE in dl 1101/1107.
1-19/97	5	G3	~ 60 % CPE in pm975 NO CPE in dl 1101
	6	G4	pm975 ✓ NO CPE dl 1101
	7		G5 pm975 ~100 % ✓ G3 dl 1101/1107 ~CPE ? - NO
	10		pm975 dl 1101/ (NO CPE yet) dl1309 (Photos)
	15		dl1101/1101 (morphology change CPE ?) G4
	24		dl 1101/ G5

EXHIBIT

I 13

Growing II

infected 1/31/97

	100 d1309	500 pfu	100 pm975	500	1 d1101/1107	100	500
Day 1	NO CPE	〃	NO CPE	〃	NO, CPE		〃
Day 2	Few cells rounded	Few cells rounded	Few cells rounded	Few cells rounded	NO CPE		〃
Day 3	10% CPE	-20% CPE	20%	50% CPE	NO CPE		
Day 6	100% CPE in pm 975; d1309						
	NO CPE in d1 1101/1107						
Day 14		—	—		500 pfu - CPE starts.		
Day 17		—	—		100 pfu - CPE starts		
					500 pfu - CPE ++		

EXHIBIT

Z-14

Growth arrested HEL 299

		Days	
1/22/91	G1	3	pm975 — -70 C
		5	pm975, dl 1101/1107, dl 309 (photos)
		7	pm975 -70°C (photos)
		10	pm975; dl 1101/1107 (NO CPE)
			(GA4) (GA9)
		14	pm975
			(GA3)
		15	-pm 975 NO CPE.
			501 cells from control & pm975. dl 1101/1101
			me came off. NO contamination.
			May be due to not changing media?
		19	pm975 (GA7)

EXHIBIT

I 15

Growing cells
on coverslips
for IF & CPE

HEL 299 cells

counted 1 dish = 188,000 cells :- (1:35 spl

(1) virus pm 925 pfu 4.06 x 10^10

for 100 pfu = ~~100~~ x 1.88 x 10^5 / 4.06 x 10^~~9~~ 2 = 1.88 / 4.0 x 10^2

dilute the virus to 10^9 titer.
50 μl to 500 μl serum free media

= 4.6 μl

(2) virus dl 1101/1107 = ~~100~~ x 1.88 x 10^5 / 1.95 x 10^~~9~~ 2 = 1.88 / 1.95 x 10^2

dilute the virus to 10^9
10 μl to 1ml medium.

= 9.6 μl

(3) +ve control dl 309 = ~~100~~ x 1.88 x 10^5 / 2.15 x 10^~~9~~ 2 = 1.88 / 2.15 x 10^2

dilute the virus to 10^9
10 μl to 1ml medium

= 8.7 μl

10 4

EXHIBIT
B16

Growth arrested HEL 299 p12 cells   p II

24 days

0	HEL 299 (Mock)	= 0
1	"	+1
2	"	−1
3	"	0
4	"	+1
5	"	−1
6	HEL 299 + dl 1101/1107	= 0
7	"	+1
8	"	−1
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
	Growing cells	3 days
21	HEL 299 mock	= 0
22	"	= +1
23	"	−1
24	+ pm 975	=
25	"	
26	"	
27	dl 1101/1107	
28	"	
29	"	
30	dl 309	
31	"	
32	"	
33	pm 975	
34		
35		
36		
37		

EXHIBIT
I 17

8/11

Growth arrested HEL299 cells infected c̄ dl1101/1107

2nd day

1	HEL 299 (mock)	= 0	TMY 400 B+W	
2	"	+1		
3	"	−1		
4	"	0		
5	"	+1		print reads 0 = 1
6	"	−1		
7	HEL299 + dl 1101/1107	= 0		
8	"	+1		
9	"	−1		
10	"	0		
11	"	+1		
12	"	−1		
13	"	0		
14	"	+1		
15	"	−1		
16	"	0		Same
17	"	+1		
18	"	−1		
19		0		
20		+1		
21		−1		

Growing 21,22,23 mock

Growing cells 3rd day

22	HEL 299 mock	= 0	
23	"	+1	
24	"	−1	
25	+ pM975	0	
26	"	+1	
27	"	−1	
28	dl 1101	0	
29	"	+1	
30	"	−1	
31	dl 309	0	Same
32	"	+1	
33	"	−1	
34	pM 975	= 0	
35	"	+1	
36	"	−1	
37	"		

EXHIBIT
I 8

Growth arrested HEL 299 cells
- Growth Curve :-

count the cells :- 347,000 cells :-

(1) 100 pfu =
for dl1101/1107 $= \frac{\cancel{100} \times 347,\cancel{000}}{1.95 \times 10^{4}} = \frac{347}{1.95 \times 10^{4}} = 17\,\mu l$

dilute the virus to 10^9
10 μl to 1 ml

(2) 100 pfu
for pm 975 $= \frac{\cancel{100} \times 3.47 \times 10^{5}}{4.86 \times 10^{2}} = \frac{3.47}{4.86 \times 10^{2}} = 8.5\,\mu l$

dilute the virus to 10^9
50 μl to 500 μl

(3) for CNTR dl 309 $= \frac{\cancel{100} \times 3.47 \times 10^{5}}{2.15 \times 10^{2}} = \frac{3.47}{2.15 \times 10^{2}} = \boxed{16\,\mu l}$
dil to 10^9
10 μl to 1 ml

1)
wash
infect
EMEM 0.2% FCS

EXHIBIT
I 19

p III photographs: Tmax 100 B/w

Growth arrested HEL299, 3 days

1	HEL299 - mock	= 0
2	"	+1
3	"	-1
4	"	0
5	"	+1
6	"	-1
7	+ pm 975	0
8	"	+1
9	"	-1
10	"	0
11	"	+1
12	"	-1
13	"	0
14	"	+1
15	"	-1
16		0
17		+1
18		-1

C

pm 975, 3rd day, 6A

Nucleus appears darker than Control

KRH -

EXHIBIT

420

p IV in photos

Growing 9th day

Tmax 100 B/W. Prints

1	HEL 299 (Mock)	2 N CO	
2	"	+1	
3	"	−1	
4	"	0	
5	"	+1	
6	"	−1	
7	dl 309	0	
8	"	+	
9	"	−1	CPE ✓
10	"	0	
11	"	+1	
12	"	−1	
13	pM 975	0	
14	"	+1	
15	"	−1	CPE ✓
16	"	0	
17	"	+1	
18	"	−1	
19	dl 1101	0	
20	"	+1	0 ?
21	"	−1	
22	"	0	0
23	"	+1	
24	"	−1	
	Growth arrested 5th day		
25	HEL 299 Mock	2 0	
26	"	+1	
27	"	−1	
28	pM 975	0	CPE — Not yet NO CPE Sign
29	"	+1	
30	"	−1	
31	"	0	
32	"	+1	
33	"	−1	
34	dl 1309	0	shows starting CPE
35	"	+	
36	"	−1	
37	"	0 less light	

EXHIBIT
I-21

8/ Photographs'

	Growth arrested HEL 288 9th day		
	Carl took 1-18 photos		
19	HEL. LA.	0	
20	"	+1	
21	"	-1	
22	pm975	0	
23	"	+1	
24	"	-1	NO CPE sign
25	"	0	
26	"	+	
27	"	-1	
28	dl1104	0	
29	"	+1	
30	"	-1	NO CPE sign
31		0	
32		+	
33		-1	
34	dl 309	0	
35	"	+	
36	"	-1	shows CPE,
37	"	~~8~~0	
38	"	+1?	

EXHIBIT I22

p6

photos:

Tmax 100 Black + white

Growth arrested HEL299 - 10th day.

1	HEL 299, GA, mock	0	
2	"	+1	
3	"	-1	
4	PM975	0	
5	"	+1	
6	"	-1	
7	"	0	NO CPE?
8	"	+1	
9	"	-1	
10	dl 1101/1107	0	
11	"	+1	
12	"	-1	NO CPE
13	"	0	
14	"	+1	
15	"	-1	
16	dl 309	0	
17	"	+1	shows CPE - growing
18	"	-1	
19	"	0	
20	"	+1	
21	"	-1	

Growing cells → 15 days

22	HEL 299 mock	0	
23	"	+1	
24	"	-1	
25	PM975	0	
26	"	+1	All the cells rounded & most of them floating (dead cells)
27	"	-1	
28	dl 309	0	
29	"	+1	All the cells rounded & floating (dead)
30	"	-1	
31	dl 1101/1107	0	
32	"	+1	Cells are longer, lengthier, some cells have ~~bigger~~ larger nuclei - CPE?
33	"	-1	
34	dl 1101/1107	0	

EXHIBIT
I23

P7

photographs 3rd day

Growing HEL 299 p17

1	HEL 299	6	2	0		
2		"		+1		
3		"		-1		
4	dl 309		100 pfu	0		
5		"		+1	100 pfu	~10 ? UPS
6		"		-1		
7	dl 309		500 pfu	0		
8			"	+1	500 pfu	~30 ?
9			"	-1		
10	pm 975,		100 pfu	0		
11		"		+1		
12		"		-1	100 pfu	~20 ?
13		"		0		
14		"		+1		
15		"		-1		
16	pm 975		500 pfu	0		
17			"	+1		
18			"	-1	500 pfu	~41 ?
19			"	0		
20			"	+1		
21			"	-1		
22	dl 1101		100 pfu	0		
23			"	+1		
24			"	-1	100 pfu	NO UPE
25		"		0		
26		"		+		
27		"		-1		
28	dl 1101		500 pfu	0		
29			"	+1		
30			"	-1	500 pfu	NO UPE
31			"	0		
32			"	+1		
33			"	-1		
34	HEL 299		CONTROL	0		
35		"		+1		
36		"		-1		

EXHIBIT

I-24

p 8 photographs

Growing 24th day:-

1	HEL 299.6	=	0		
2	"		+1		
3	"		-1		
4	"		0		
5	"		+1		
6	"		-1		
7	dl 1101/1107		0		
8	"		+1		
9	"		-1		shows 0
10	"		0		
11	"		+1		
12	"		-1		
13	"		0		
14	"		+		
15	"		-		

GA HEL 299 19 days

16	HEL 299 GA	-	0		
17	"		+1		
18	"		-1		cells are dying
19	"		0		
20	"		+1		
21	"		-1		
22	pM 425		0		
23	"		+1		
24	"		-1		
25	"		0		
26	"		+1		
27	"		-1		
28	dl 1101/		0		
29	"		+1		
30	"		-1		
31	"		0		
32	"		+1		
33	"		-		
34	HEL 299 C		0		
35	"		+1		
36	"		-1		

EXHIBIT

R-25

Expt 3 Growth arrested 4Z-299 cells p17 [redacted]

– Growth Curve :–

Count the cells : 347,500 cells

(1) 10 pfu = for dl1101/1107 $= \frac{10 \times 347,500}{1.95 \times 10^{4}} = \frac{347}{1.95 \times 10^{4}} = 17\mu l$

dilute the virus to 10^{7}

$1\mu l$ to 1 ml

500 pfu = 85 μl

(2) 100 pfu for pm975 $= \frac{100 \times 3.47 \times 10^{5}}{4.06 \times 10^{2}} = \frac{3.47}{4.06 \times 10^{2}} = 8.5\mu l$

dilute the virus to 10^{9}

50 μl to 500 μl

500 pfu : 42.5 μl

(3) for CNTR dl309 $= \frac{100 \times 3.47 \times 10^{5}}{2.15 \times 10^{2}} = \frac{3.47}{2.15 \times 10^{2}} = 16\mu l$

dil to 10^{9}

10 μl to 1 ml

500 pfu = 80 μl

EXHIBIT 826

↓
wash
infect
EMEM 0.2% FCS

Days 1, 2 = NO CPE

3/10/97 F Days 3 : dl1101, pm975, dl309 NO CPE / 100 pfu
At 500 pfu, ē pm975 & dl309 shows beginning of CPE

Day 4 } = NO CPE 1101/1107 (100 & 500 pfu)
Day 5 } NO CPE pm975 / 100 pfu
= CPE begins pm975 500 pfu only

Day 6 = NO CPE pm975 100 pfu :

3/13/97 Day 7 = NO CPE dl1101/ 100 & 500 pfu
NO CPE dl309 100 pfu. | CPE in dl309 500 pfu

Growth curve :- Frozen :- days :

KEL- 299

	pm975,	dl 1101
Growing:	1,3,5,6,7,	1,3,5,6,7, 10, 15, 19
GA :	3, 5, 7, 10, 14	5, 7, 10 19

GA

pm975 GA :
√GA 3 = 14th day
√GA 4 = 10th
~~GA 2 = 5th~~
√GA 7 = 19
~~GA 1 = 3rd~~

Growing
√2G = 3rd day 67
√3G = 5th "
√4G = 6th " 64
√5G = 7th "

GA

dl 1101/1107 :
√GA7 = 19th day
√GA9 = 10th "
√GA2 = 5th
√GA 23 = 23 day

Growing
G3 = 5th day
G4 = 15th
G24 = 24th day

2/18/97

pm975: GA = 10, 14, 19 day

Growing = 3, 5, 6, 17 day

3 or 7?

Growth Curve :- Plaque assay:

293 cells :-

Growing : pm 975 : days 1, 3, 5, 6, 7] # 9

GA : pm 975 : 3, 5, 7, 10.

Growing : dl1101 : 1, 5, 7, 10, 15] # 7

GA : 7, 10

dl309 :

infections = ~~0.5 ml~~ und.
~~0.1 ml~~ 10^{-2}
10^{-4}
10^{-5}

DO pm 975 first

# of points Growing = 5 = 5x = 25 dish
GA = 4 = 4x = 20 dish
35

0.1 ml → 9.9 ml 10^{-2} → 0.1 ml → 9.9 10^{-4} → 0.1 ml → 0.9 10^{-5} → 0.1 ml → 0.9 10^{-6}

~~0.5 ml adsorption~~:
1 ml 10^{-2} to 10^{-6}

0.1 ml und.

Stain on 2/24/97

EXHIBIT
228

40 dishes : x5 = ~~100~~ 200 ml | 200 ml
x2

(A) ~~50 ml~~ 2x DMEM = 50 ml
7.5 % Sod. HCO_3 = 5 ml
FCS = 2 ml

(B) 1.8 % Agarose = 43 ml

dl 1101/1107 Growth Curve: plaque assay

dl 1101/1107

GA:

	Day
GA7 =	19
GA9 =	10
GA2 =	5
GA =	23

Grown

G3 = 5th

G4 = 15th day

G24 = 24 hr

OA3 pa925

Dilutions

0.1 ml → 9.9 ml (10^{-2}) → 0.1 ml → 9.9 ml (10^{-4}) → 0.1 → 0.9 (10^{-5}) → 0.1 ml → 0.9 (10^{-6})

↓

0.5 ml infection:

EXHIBIT

229

GA pm 975 5^{th} day.

31×10^4 / 0.5 ml

62×10^4 / 1 ml

6.2×10^5 / ml pfu →

Growth Curve 3/4/97

d1 1001/1007 100 pfu

- Growing Small plaques wait one more day -

G3 = 5th day . 1.4 x10^4
15th day = 10^{-2} = 406 / 1ml x10^2 52
= 4 x10^4 57
64
60
24th day = 10^{-4} 66 / 0.5ml 233 /0.5

132 / 1ml

= 132 x 10^4

= $\boxed{1.3 \times 10^6}$ /ml

GA

5th day = 1.1 x 10^3 pfu/ml. (No plaques in ud.)
10th day = 7 x 10^2 / 0.5ml = 14 x 10^2
= $\boxed{1.4 \times 10^3}$

19th day = 42 plaques / 0.5ml (small)
= 84 / 1ml x 10^2

= 8.4 x 10^3 pfu/ml

23rd day = 28 x 10^{2} / 0.5ml
= 56 x 10^2
= 5.6 x10^3 pfu/ml

EXHIBIT
I 30

Growth Curve: 2-25-97

Growing pm975: 180 pfu

		plaques	pfu/ml
3rd day	10^{-6}	71	7.1×10^{7}
5th day	10^{-6}	13	1.3×10^{7}
6th day	10^{-6}	11	1.1×10^{7}
7th day	10^{-6}	7	7×10^{6}

GA

			pfu/ml
10th day:	10^{-4}	12	1.2×10^{5}
14th day	10^{-4}	32	3.2×10^{5}
19th day	10^{-4}	18	1.8×10^{5}

GA HEL 299 infected c̄ pm975 did not CPE, but it gave viral yield ~ 10^{4}

5th day GA pm975 is plaque assayed today 2/25/97

EXHIBIT

I 31

Growth Curve:

3/5/97

pm 975 - growing 1 + 3rd lag.

~~dilu? ??~~ 5th

0.1ml 0.1ml 0.1ml 0.1ml

9.9 9.9 0.9 0.9

10^{-2} 10^{-4} 10^{-5} 10^{-6}

Growth Curve: plaque assay

3/11/97

Growing: G1 day dl 1101/1107 ; G1 dl 309

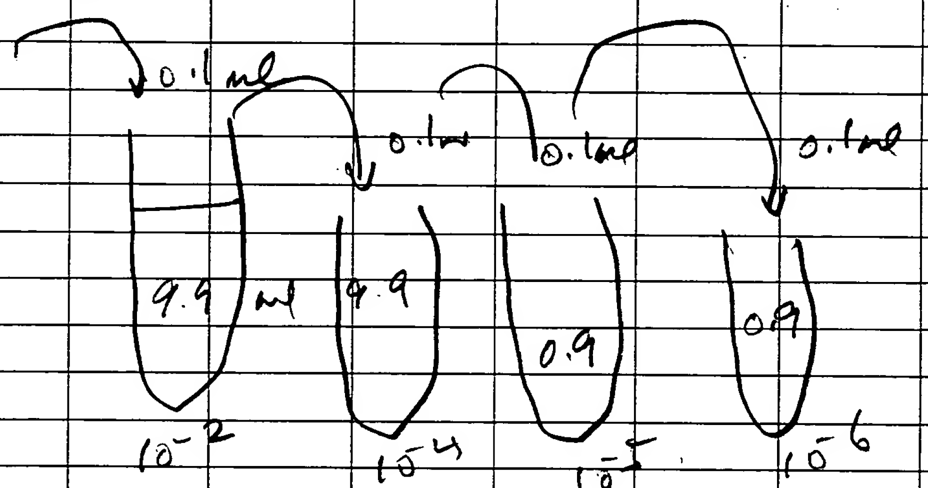
G2 (3 day) " G5 dl 309

GA = GA1 dl 1101/1107 ; GA1, dl 309

GA5 day - dl 309

7 viruses :

dilutions



0.5 ml

St. S. Grand; Amoco

867-6?92

782-1089

?84-3?3?

EXHIBIT

I 33

Growth Curve.

3.11.97

Growing :- Day 1 (G2) pu 975

$= 4 \times 10^{-4}$ 0.5ml

$= 4 \times 10^{4}$ $= 8 \times 10^{4}$/ml

6th day infection

Day 3 $= 19 \times 10^{-5}$

1×10^{-6}

$= 1.9 \times 10^{6}$ wait 2 more days.

0.5ml

3.8×10^{6}/ml

very small plaques. allow two more days to form more plaques.

31×10^{6} (Counted after 2 days)

3.1×10^{7} Pfu/ml

EXHIBIT

P 34

Growth arrested   3/31/97

500 pfu

HEL 299 = # cells 351,000

1) for dl 1101/07 = $\dfrac{\cancel{500}\,5 \times 351,\cancel{000}}{1.95 \times \cancel{10^9}\,\cancel{10^7}\,10^4} = \dfrac{5 \times 351}{1.95 \times 10^4} = 90\ \mu l$ ✓

dilute virus to 10^9

(10 µl to 1 ml)

2) for dl 309 = $\dfrac{\cancel{500}\,5 \times 351,\cancel{000}}{2.15 \times \cancel{10^9}\,\cancel{10^7}\,10^4} = \dfrac{5 \times 351}{2.15 \times 10^4} = 81\ \mu l$ ✓

dilute the virus to 10^9

(10 µl to 1 ml)

↓

~~wash~~

infect, remove,

add ~~EMEM~~ + 0.2% FCS.

Freeze) 1 hour 3/31/97 ✓

1 day

6

~~8~~ 10

15

~~6~~

10

15

21

EXHIBIT

235

CPE Table

	Growing (100 pfu/cell)			HEL 299 - p[17]
	dl309	pm975	dl1101/1107	
Day				
1	−	−	−	
3	+	+	−	
5	+++	+++	−	
6	++++	++++	−	
7			−	
10			−	
15			±	(cell morphology changes)
24			++	

Growth arrested

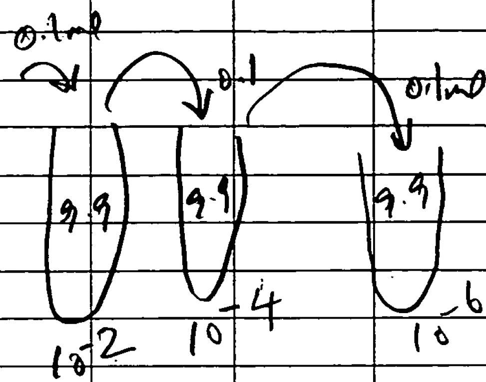
Day	dl309	pm975	dl1101/1107
1	−	−	−
3	±	−	−
5	+	±	−
9	++	−	−
10	+++	−	−
19		±	−
24		±	−

5/22/97

Titer: 293 cells · dl 309 GA MEL [illegible]

dl 309 GA: 800 pfu/ml

dilutions



3hr, 1d, 4, 6, 8 days.
1 6 8 15

EXHIBIT
I37

5/14/97
5/15/97
5/19/97

HEL 299 cells

500 pfu/cell

Growing

	dl 309	dl 1101/07	
3 hrs	9×10^3	2×10^4	–
1 day	1×10^4	4.2×10^4	–
4 "	1.6×10^7	1.2×10^5	–
8 "	$6^{th} \rightarrow 2.3 \times 10^7$ $\rightarrow 9 \times 10^6$	1.4×10^5	–
10 -		$2.4 \times 10^5 \rightarrow$	7.1×10^5
15 "		$2.5 \times 10^5 \rightarrow$	2.2×10^6
21 "		$3 \times 10^6 \rightarrow$	4.6×10^6

Growth arrested:

3 hrs	8.9×10^3	4×10^4	
1 day	6.1×10^4	4.4×10^4	
4 "	6.2×10^5	4×10^4	
8 "	$6^{th} \rightarrow 1.3 \times 10^6$ $\rightarrow 9.2 \times 10^6$	5×10^4	5.2×10^4
10	–	$1. \times 10^4$	1.6×10^4
15 -	5×10^5	1×10^4	1.4×10^4
21		2×10^4	2.2×10^4

= 9 * 10 ^ 3

#24 Mohan World Book(?)

7/12

① harvest flasks p 150 [ad I / ad III / Bailey

② freeze 11-Q - ⑥ | split into (p 100)
② 11-N - ⑥ 11-N - ③
11-M - ③

A549 MT2 hygro (freeze)
M56 - CrmA ② (Freeze + split) (p.100)
11.6.11 (split 1:3) just a bit too heavy
CrmA - 11.6 ⑤ split / freeze
11.6 - CrmA ⑨ split / freeze

Bring up M56 - crmA$_2$ to FLASK

3. HARvest virus spinner

4. split other spinners
My Spinner:

— 5.4 x 10^5 cells / ml
split ½ — freeze cells

— 4.4 x 10^5 / ml / 1 liter removed and frozen

EXHIBIT
K1

8 - 100MM

7/13

todo

phone:

Ab in White/red File on Ams desk

Inventory virus book * [illegible] / + [illegible]

(K.BS from freezer) / when brought up

3day infection use [illegible] 7001

2×10^5/ml

Develop the gel (Monday)

7.5×10^7

~~(cells frozen + thrown out)~~ ✓

~~no less than 2×10^5/ml~~

3mls / 250mls cell

([illegible] / media)

Ad 1 + Ad 6 virus stock (5mls of stock)

Set up 1-100ml → flask media.

11.6 pre-Absorbed. ✓ Set up

grow up

293 cells from Kaye (Dupe [illegible]) OK

Ginsberg H5dl 111 (earliest stock) (175 flasks)

V5103 5-2-1 stock 50ul → 100mm plate → flasks

V9103 5-4-1 spun down / media [illegible] 2% FBS 100ul → 100mm plate

(harvested)(960725)

Bayley 11/07/6 (911003) 104-8 loc 50ul → 100mm plate

freeze thaw

harvested (960725) 104-8

EXHIBIT

K2

1. spinners 1 liter K.B's
Count 3.4×10^5 cells/ml
split 500mls into a 3 liter flask add 1.5 liter Joklik's media. Bring original 1 liter spinner up to 1 liter (Jokliks + 10% hrs) serum)

2 3 liter spinner of K.B's infected
Count is 3.2×10^5 cells/ml
- Infect with (Baileys) 11/07/6 911003)

Baileys used was of a new stock grown up in 293 cells. last week Baileys. 9602.
(Titer unknown) infected at 11:30 → 12:30

used 20mls of Virus into 1 liter of cells for 1 hour. then added back media
(2 Liters Jokliks + 100 ml denatured Horse serum)

3. develope gels.

4. split 293 give one plate to Ann. (split other cells)

5. look at plaques that are forming.
Very few new since yesterday
Count tomorrow.

EXHIBIT
K3

1. Split 293. A549 in 60mm (dishes)

2. Bring 3liter spinner up to add 1 liter
Count. 4.2×10^5

infect tomorrow w/ 704

3. New media into all 56-29, 56-25, M56-2MD, M56-3MD, CrmA-56-3, 11.6-1MD, 11.6 CrmA 2 +
~~A549/1-Q~~

---

4. Harvest infected spinner; Bailey, wash pellet w/ PBS 2x (cold) resuspended it into 24 mls total vol of .01 M Tris ph 8.0
mls of cells + tris = 24 mls
put into snap cap tubes + freeze at -70°.
put in freeze (right inside Lindas lab)

5. Split + freeze ~~250~~ - 500mls of KB back up spinner.

6. Baileup
cells are not completely infected some are dying so must harvest cells

EXHIBIT
K4

CsCl Banded the virus Ad1 960825
1101/1107 Baileys (960902)

1 freeze (thaw)
2 Sonicate x 2
3 Vol after son 1101 < 21mls
Ad1 < 19mls

add $V \times 0.51 = g$ of CsCl

10.71g in 1101/1107 Baileys
9.69 g in Ad1.

infect 3 liter Spinner with
dl 717 Vp 891208 Titer 1.3×10^{11}

Cell count 3liter 3.5×10^5
Vol = 3liters (3000mls)
$3.5 \times 10^5 \times 3000$mls 10.5×10^8 total cells

use 20 pfu's / cell

.162ul = $\dfrac{.0105 \times 10^{11}}{1.3 \times 10^{11}} \times 20$ pfu's
use

KB's are healthy no clumping

EXHIBIT
K5

Black (first)

960 — 9/25 Plaque Assay

	Virus	Dil	A	B	C	comment
1.	1101/1107 (first infection)	.5x10^{-6}	9	25	—	
		.5x10^{-7}	3	1	—	
		.5x10^{-8}	Ø	Ø	Ø	
		.5x10^{-9}	Ø	Ø	Ø	
		.5x10^{-10}	Ø	Ø	Ø	
2	765	.5x10^{-6}	TMTCA			
		.5x10^{-8}	3	Ø	Ø	
		.5x10^{-9}	Ø	Ø	Ø	
		.5x10^{-10}	Ø	Ø	Ø	
3	717	.5x10^{8}	Ø			Very hard to see plaques. today
		.5x10^{9}	Ø	Ø	Ø	
		.5x10^{10}	Ø	Ø	Ø	

EXHIBIT

K6

1101 / 1107	A	B	C	Comments
$.5 \times 10^{6}$	TMTCA	TMTCA		
$.5 \times 10^{7}$	42	63		
$.5 \times 10^{8}$	6	4	8	small plqs. (flat)
$.5 \times 10^{9}$	1	Ø	1	
$.5 \times 10^{10}$	Ø	[illegible]	Ø	

717	A	B	C	Comments
$.5 \times 10^{-6}$	TMTCA			
$.5 \times 10^{-8}$	TMTCA			hard to count because everywhere (very faint) very small
$.5 \times 10^{9}$	34	28	36	
$.5 \times 10^{10}$	1	3	3	

765	A	B	C	Comments
$.5 \times 10^{-6}$	TMTCA			
$.5 \times 10^{-8}$	12	19		
$.5 \times 10^{-9}$	2	1	1	
$.5 \times 10^{-10}$	Ø	Ø	Ø	

EXHIBIT

K7

1101/1107	A	B	C	Comment
$.5 \times 10^{-6}$	TMTC	TMTC		start off small grow very quickly
$.5 \times 10^{-7}$	9	14		
$.5 \times 10^{-8}$	14	14	12	
$.5 \times 10^{-9}$	∅	∅	∅	
$.5 \times 10^{-10}$	∅	—	∅	
717				
$.5 \times 10^{-6}$	TMTC			
$.5 \times 10^{-8}$	TMTC			
$.5 \times 10^{-9}$	59	45	55	Very very small
$.5 \times 10^{-10}$	4	4	8	
765				
$.5 \times 10^{-6}$	TMTC			
$.5 \times 10^{-8}$	9	11		
$.5 \times 10^{-9}$	2	3	3 monolayer strong	
$.5 \times 10^{-10}$	1	∅		

EXHIBIT

L8

5

1101/1107	A	B	C	Comment
.5x10-6	TMTC	mold + TMTC		
.5x10-7	all together hard	to count		
.5x10-8	5	7	2	
.5x10-9	Ø	Ø	Ø	
.5x10-10	Ø	Ø	Ø	
717				
.5x10-6	TMTC	—	—	
.5x10-8	TMTC	TMTC	—	small but visible
.5x10-9	14	13	7	
.5x10-10	5	4	10	
765				
.5x10-6	TMTC			
.5x10-8	5	3		
.5x10-9	1	2	3	
.5x10-10	1	mold	1	

EXHIBIT
K9

1101/1107	A	B	C	Comment
.5x10^-6	TMTC			
.5x10^-7	TMTC	TMTC	TMTC	
.5x10^-8	no new areas Ø	Ø	Ø	
.5x10^-9	Ø	Ø	Ø	
.5x10^-10	Ø	Ø		
717				
.5x10^-6	TMTC			
.5x10^-8	TMTC	TMTC		
.5x10^-9	TMTC	TMTC	TMTC	
.5x10^-10	3	Ø	2	
765				
.5x10^-6	TMTC			
.5x10^-8	Ø	2	monolayer gone	
.5x10^-9	Ø	Ø	Ø	
.5x10^-10	Ø	throw out	Ø	

EXHIBIT

K10

1101/1107	A	B	C	Comment
$.5 \times 10^{-6}$	TMTCA			plq came up quickly then tapered off
$.5 \times 10^{-7}$	7	2		
$.5 \times 10^{-8}$	3	5	6	
$.5 \times 10^{-9}$	Ø	Ø	Ø	
$.5 \times 10^{-10}$	Ø	Ø	Ø	
717				
$.5 \times 10^{-6}$	TMTA	TMTA		
$.5 \times 10^{-8}$	TMTA			
$.5 \times 10^{-9}$	16	17	19	
$.5 \times 10^{-10}$	3	4	7	
765				
$.5 \times 10^{-6}$	TMTCA			
$.5 \times 10^{-8}$	5	3		
$.5 \times 10^{-9}$	1	Ø	Ø	
$.5 \times 10^{-10}$	Ø	Ø	Ø	

1101/1107	A	B	C	Comment
.5x10^{-6}	TMTC	TMTC	—	
.5x10^{-7}	TMTC	TMTC	—	
.5x10^{-8}	+1	+2	+0	
.5x10^{-9}	+0	0	+0	
.5x10^{-10}	0		0	
717				
.5x10^{-6}	TMTC	—	—	
.5x10^{-8}	TMTC	—	—	
.5x10^{-9}	TMTC →			
.5x10^{-10}	+0	+2	+0	
765				
.5x10^{-6}	TMTC	—	—	
.5x10^{-8}	+1	+1	—	
.5x10^{-9}	+0	+0	+0	
.5x10^{-10}	+1	+0	—	

EXHIBIT

K12

1101/1107	A	B	C	Comment
$.5 \times 10^{-6}$	mold	VI 34		
$.5 \times 10^{-7}$	TMTC	blended together TMTC		
$.5 \times 10^{-8}$	5	4	9	Really big
$.5 \times 10^{-9}$	Ø	Ø	Ø	
$.5 \times 10^{-10}$	Ø	Ø	Ø	
765				
$.5 \times 10^{-6}$	TMTC			
$.5 \times 10^{-8}$	TMTC			
$.5 \times 10^{-9}$	4	3	12	monolayer looks bad
$.5 \times 10^{-10}$	Ø	Ø	Ø	no new just bigger
768				
$.5 \times 10^{-6}$	TMTC			
$.5 \times 10^{-8}$	TMTC	TMTC		
$.5 \times 10^{-9}$	TMTC	TMTC		
$.5 \times 10^{-10}$	3	Ø	2	

EXHIBIT
K13

α-7/Vesla

10

		A	B	C	comment
760	$.5\times10^{-8}$	TMTC	TMTC	TMTC	
	$.5\times10^{-9}$	37	29	26	
	$.5\times10^{-10}$	3	1	4	
327	$.5\times10^{-8}$	TMTC	TMTC		
	$.5\times10^{-9}$	60	74	74	
	$.5\times10^{-10}$	18	17		
1101/1107	$.5\times10^{-6}$	TMTC	TMTC		
	$.5\times10^{-7}$	TMTC	TMTC		
	$.5\times10^{-8}$	TMTC	TMTC		
	$.5\times10^{-9}$	66	64	70	Big plqs
	$.5\times10^{-10}$	8	7		
702	$.5\times10^{-8}$	TMTC	TMTC		
	$.5\times10^{-9}$	TMTC	TMTC	TMTC	
	$.5\times10^{-10}$	36	30	36	

EXHIBIT
K14

		A	B	C	comment
760	.5x10^{-8}	TMTC	TMTC	TMTC	
	.5x10^{-9}	44	40	42	
	.5x10^{-10}	5	4	6	
327	.5x10^{-8}	TMTC	TMTC		
	.5x10^{-9}	64 really TMTC	74	72	
	.5x10^{-10}	18	16	37 Missed counting first time	
1101/1107	.5x10^{-6}	TMTC			
	.5x10^{-7}	TMTC			
	.5x10^{-8}	TMTC			
	.5x10^{-9}	25	21	22	med nice plq.
	.5x10^{-10}	11	9		
702	.5x10^{-8}	TMTC	TMTC		
	.5x10^{-9}	~~[illegible]~~ almost TMTC 21	24	22	
	.5x10^{-10}	15	14	15	

EXHIBIT

KIS

a:'/Nasha

		A	B	C	comment
760	.5 x 10^{-8}	TMTC	TMTC	TMTC	
	.5 x 10^{-9}	10	20	15	small
	.5 x 10^{-10}	1	3	1	
327	.5 x 10^{-8}	TMTC	TMTC	TMTC	
	.5 x 10^{-9}	TMTC	TMTC	TMTC	
	.5 x 10^{-10}	10	8	9	
1101/1107	.5 x 10^{-6}	TMTC	—		
	.5 x 10^{-7}	TMTC	TMTC		
	.5 x 10^{-8}	TMTC	TMTC		
	.5 x 10^{-9}	4	5	4	
	.5 x 10^{-10}	Ø	Ø		
702	.5 x 10^{-8}	TMTC	TMTC		
	.5 x 10^{-9}	TMTC			
	.5 x 10^{-10}	3	3	5	

EXHIBIT
K16

2 nm/[illegible]

		A	B	C	Comments
760	.5 x 10^-8	TMTCA	TMTCA		
	.5 x 10^-9	TMTCA	TMTCA	3	Very small easy to count.
	.5 x 10^-10	4	3	1	
327	.5 x 10^-8	TMTC	TMTCA	—	
	.5 x 10^-9	TMTC	TMTCA	TMTCA	
	.5 x 10^-10	3	5	6	
1101/1107	.5 x 10^-6	TMTCA	—		
	.5 x 10^-7	TMTCA	TMTCA		
	.5 x 10^-8	TMTCA	TMTCA		
	.5 x 10^-9	0	1	0	Very acidic
	.5 x 10^-10	2	1	0	
702	.5 x 10^-8	TMTCA			
	.5 x 10^-9	TMTCA	TMTCA	TMTCA	Very close hard to count
	.5 x 10^-10	1	∅	1	

EXHIBIT
47

[redacted] 2nd/Washes

		A	B	C	COMMENT
760	$.5 \times 10^{-8}$	TMTC	TMTC		
	$.5 \times 10^{-9}$	TMTC	TMTC		
	$.5 \times 10^{-10}$	3	[illegible]	2	
327	$.5 \times 10^{-8}$	TMTC	TMTC		
	$.5 \times 10^{-9}$	TMTC	TMTC	TMTC	
	$.5 \times 10^{-10}$	8	5	2	
1101/1107	$.5 \times 10^{-6}$				all plates too acid no new colonies
	$.5 \times 10^{-7}$				
	$.5 \times 10^{-8}$				
	$.5 \times 10^{-9}$				
	$.5 \times 10^{-10}$	∅	∅	∅	
702	$.5 \times 10^{-8}$	TMTC (throw out)			
	$.5 \times 10^{-9}$	TMTC	TMTC	TMTC	throw out
	$.5 \times 10^{-10}$	∅	∅	∅	

Keep plates 5×10^{-10} 760, 327
still growing plpa

EXHIBIT
K18

Plaque Assays (% of final plaque #)

	Virus	5d	7d	12d	13d	16d	19d	25d	30d
1	1101/1107	0.	18.3	55.	67.9	80.7	80.7	83.5	100.%
2	sub765	2.6	32.8	57.8	65.5	79.3	81.	83.6	100.
3	dl 717	0.	10.3	33.8	54.4	82.4	89.7	92.6	100.

titer of 1101/1107 : $6.61 * 10^9$ /ml
(harvested from KB cells at day 2)

10-15-96 Plaque Assays (% of final plaque #)

	Virus	8d	10d	13d	16d	21d
1	pm760	32.4	78.	94.2	97.7	100.%
2	dl 327	32.1	65.4	82.1	90.7	100.
3	dl 1101/1107	65.7	94.6	98.7	100.	100.
4	dl 702	64.2	91.8	98.7	100.	100.

titer of 1101/1107 : $1.95 * 10^{11}$ pfu/ml
(harvested from KB cells at day 3)

EXHIBIT
1019

EXHIBIT
K20

#	Virus	From	INF	Harvest	Spin	Plaq	Comments			freezer lo
1.	Ad4Δ19K	(VS58)	961016	961018						
2.	Ad4ΔE3	(961055)	961104	961106						
3.	Ad4ΔE3B	(VS56)	961031	961102						
4.	1101/1107	(Baileys)	960905	960907	✓	✓				1-1
5.	1101/1107	2nd batch	960917	960919	✓	✓				1-1
6.	dl 702	(VS33)	961002	V2day	✓	✓				1-2
7.	dl 773	(VS230)	960909	V2day	✓					101-5
8.	dl 717	(VS118)	960906	960908	✓	✓				2-2
9.	327	(VS212)	960914	960917	✓	✓				5-5
10	~~dl~~ 760	(VS227)	960915	960917	✓	✓				4-6
11	Ad 5	(VS198)	960907	960908	✓	✓	Great bands			101-2
12	Ad 1	(sup)	960905	960907	✓	✓				2-1
Repeat 13	dl 762	(Repeat)	96~~1008~~	96~~1008~~	✓	✓	Bad	No plaques		2-3
14	Ad 11	Sup	961030	961002						
15	dl 797	(VS243)	96~~1022~~ 1012	96~~1024~~ 1015						
Repeat 16	Ad 3						first spin			
17	Ad 6	SUP	960831	960803	✓	✓	Good bands			101-2
18	Ad 9	(A549 sup)	970209							
19	dl 703	VS50	961001	961003	✓					
20	dl 704	(VS167)	960802	960804	✓	✓	thin bands			5-2
21	dl 748	(VS181)	960708	960810	✓	✓				13-6
22	dl 798	(VS188)	960708	960710	✓	✓	Good			13-6
23	dl 763	(VS214)	960703	960705	✓	✓	~~Bad~~ Bands look good			2-3
24	dl 765	(VS225)	960824	960826	✓	✓				101-8
25	dl 739	(VS223)	921023	just plq	✓	✓				
26	dl 754	(VS243)	940202	just plq	✓	✓				
27	dl 712	?	950503	—	✓	✓	Plaqs Good			
28	7001	?	960724	960726	✓	✓				
29	dl 250	sup	950616	950618	✓	✓	Big plaques / nice bands			
30	Ad 41	Sup/293	(293)	→						
31	Ad 40	Sup/293	(293)	→						
32	Ad 707	(SB423 sup)	961008	961110						
33	~~Ad~~ 740	VS240	930612				~~Sep~~			
34	dl 728	(Apa24)	960120	961122			from 940620	See page		
35	Rad KS	96 VS231	970305		✓					
36	Ad 11		970203	970205	✓	✓	Great bands			
37	Ad 11		970213	970215	✓	✓	Great "	!		

4/22
Chris

Low affinity Nerve growth receptor = .1ug/ul

Viruses from Kostaya: grow in KB's first A549 then KB.

= Viruses from Bowling = grow in A549 first then then in KB! 2 flasks

+ set up 2 flasks for bowling virus. pME116 ΔΔΔ

= KB's ready to infect in 3 liter spinner

+ 1 liter bring up to 1300 liters split tomorrow.
counts

dl 707 : 1.57×10^{10}

count 3.3×10^5 x 9.9×10^8 cells/3liter

$\frac{.09.9 \times 10^8}{1.57 \times 10^{10}}$ x 10 pFU'S = .63 ul
x 5 pFU'S 315

+ Infect.

EXHIBIT
K21

4/28/monday

1. set up A549 for virus : from Costa

2. split MCF-7 for transfection of
Nerve growth factor.

3. change media on MCF-7 - transfections
11.6. - MCF-7 ~ 5-6 Clones per dish
M56 - MCF 7 ~ ~~3~~-4 " "
except for one dish which has alot more

Tues.

set up transfection W/
Low Affinity Nerve growth factor = 3 disks
" " " + 11.6 = 3 dishes
2 controls

use new prep.

EXHIBIT
K22

Coverslips of MCF-7.
1 shos Affinity Nerve growth factor .2 ug/ul
use
3:00
3:30
solution
A = (pK10g FRMN) .2ug/ul = 25ul/dish
NGF ONLY
3 NGF only
A = 75ul DNA + 800 ul opti DMEM =
B = 36ul lip + 300 ul optiDMEM
A+B for 30 min 2.4 mls opti
NGF + 11.6
3:15
A 5.7 11.6 + 75ul + 300ul opti DMEM
B. 36ul lip + 300 ul opti DMEM
A+B for 30min 2.4mls opti

2 dishes not transfected = for control
- mix A+B let set R.T for 30 min, Add Raman - media
- 1ml per dish / for five hours,
- then add 20% media (1ml) let incubate O/N
- stain tomorrow.

1 dish pMT2 2.2 + 100 opti DMEM
2:00 12 lipo + 100 opti DMEM OK
800 ul
OK Add 1ml 20% on transfection / Add 2% to infection
OK Set up flasks 293 (110 virus)
(111)
Set up 4 A549 plates 100ul to pre absorb ab
OK Add Costygas virus to flasks - put
split KB's

EXHIBIT
1623

5/2 Friday

- FREEZE FAS cells - pool
- V infection.
- split all cells/
- split KB's back

5/6

- split A549 to two flasks - for Kostya
- Clones picked
- Infect the other ~~spinner~~
-

45×10^5 cells/ml / x 3000 mls =

13,500 cells.

cells look ok: added 5 mls of the virus 45.5 from Kostya to the spinner.

(Tomorrow pick clones.)
get cells from Mohan
make Joklik's media /. ok

EXHIBIT
K24

1:30

5/13

797 ?

clear liquid freeze + thaw & spin to CS prep viruses

20 mls	KD-1 1101/1107 ADP	970508
19 mls	PME 11.6 AAA	"	970508
20 mls	dl 707	"	970420
19 mls	dl 751	"	970324

Viruses in warm room x 2 thaw,

~~glutamine also~~

20 mls dl 707 970315

split A549 cells to Flasks FOR 797 + others 1, total -

IN Freezer need Sonicaled in Beaker

location

PME = 1, 7
KD = 9, 3
751 = 11, 5
707 (970315) 8, 2
707 = 6, 12

set machine on 4° / 35K for O/N

2 1/2

2 1/2

147

EXHIBIT K25

5/14

Viruses taken out of Centrafuge

				harvest Down	
(970508)	(46-4) KD-1 (Kostya) 1101/1107 ADP:			~ 4 mls	(small thin bands)
(970324)	dl 751	(nice thick bands) good	=	≈ 4 mls	
(970315)	dl 707	(very thick big bands great)	=	≈ 9 mls	
(970423)	dl 707	(nice bands) good	=	≈ 6 mls	
(970503)	PME-11.6 AAA (BLY ADP-AAA)	(Very nice bands) good	=	~5 mls/3	

Note Don't dilute KD-1 very much got alot of extra liquid w/ virus band ~ 2 mls of actual virus.

Dilute with TSG ~ 5x — 10x

			tubes	LOC
KD-1 dilute	bring up to	10 mls	5	4-4
dL 751	bring up to	(16 mls)	8	Tray 2-5 / 2-4
dl 707 (970315)	bring up to	= (24 mls)	12	4-3? / 4-2
dl 707 (970423)	bring up to	(16 mls)	8	4-3? / 4-3
PME-11.6 ~~AAA~~ = BLY (ADP-AAA)	bring up to	(14 mls)	7	4-4

(plaque assay ~~viruses~~) 751 original 53-3/60-B
one tube of each in my box to plaque 707 original

need to

Infect 3 liter w/ H5 dl 110 / H5 dl 110 / dl 205 from sup flasks A549.

3.

EXHIBIT
K26

5/20/97

agar assay (Titer virus)

dl 751 (970324)

dl 707 (970315)

dl 707 (970423)

PME - 11.6AAA (BLY-ADP-AAA)(970503)

KD-1 (970508) (Kostya)

dilute all viruses 10ul to 10mls (10^{-3} dilution)

10ul → 10ml (10^{-3}) → 10ul → 10mls (10^{-6}) → 20ul → 2ml (10^{-8}) → 200ul → 1.8ml (10^{-9}) → 200ul → 1.8ml (10^{-10})

2 - 10^{-8}
3 - 10^{-9}
3 - 10^{-10}
] each

50 ~~200~~
5
2
1
43

100 mls 2x DME
10 mls Sodium Bi
4 mls FBS
2 mls Glut
86 mls AGAR

45
5
20.5

ADD virus to the 3liter spinner — dl 110 virus

3 liter Count: 4.2 x 10^5 cells/ml. use 1ml cells. dl 110

1 liter Count: (2.7 x 10^5 cells/ml) split.

EXHIBIT
K27

Second 5/23		A	B	C	6/2 A	B	C
751	5×10^{-8}	TMTC	TMTC	/	~~TMTC~~	TMTC	
	5×10^{-9}	10	bact	10	9	5	10
	5×10^{-10}	2	Ø	1	3	Ø	Ø
707 (very tiny)	5×10^{-8}	~~14~~	15	/	68	47	
	5×10^{-9}	2	5	2	8	2	Ø
	5×10^{-10}	Ø	Ø	Ø	Ø	Ø	Ø
707	5×10^{-8}	13	15	/	30	17	
	5×10^{-9}	3	Ø	Ø	2	3	Ø
	5×10^{-10}	Ø	Ø	3	Ø	Ø	Ø
KD-1	5×10^{-8}	Total 162	163	~~162~~	TMTC	TMTC	
	5×10^{-9}	Total ~~46~~	total ~~39~~	total ~~17~~	3	1	1
	5×10^{-10}	1	Ø	Ø	1	Ø	2
						8.1×10^{9}	
PME (nice size plaques)	5×10^{-8}	74	78	/	TMTC	TMTC	
	5×10^{-9}	16	11	20	12	14	7
	5×10^{-10}	4	3	2	1	Ø	2

EXHIBIT K28

WI 38 infections with 1101/1107 and dl308 11/24

MOI dividing quiescent 549

0.1

1

10

100

16 35 mm WI 38

8 35 mm A549 2.5 x 10^5 cells/dish

infections 11/25

1.84 x 10^?

1101/1107 (960917)

3.8 x 10^?

1/1? 2 x 10^? cells

5 x 10^4	0.1	MOI
5 x 10^5	1	
5 x 10^6	10	
5 x 10^7	100	

3.8 x 10^?

10x dil 6 µl

~~312~~

change medium on WI 38 to 0.2% FCS

11/28 infect starved WI 38 with

d308 (950511)

100

10 MOI

1

0.1

1101/1107/(960917)

100

10 MOI

1

0.1

EXHIBIT

4

BEST AVAILABLE COPY

ADP Western

infect A549 with 303, KD1 12/9

10^6 cells / dish 8.09 x 10^{10}/ml 303 ⇒ 8.09 x 10^7/μl

500pfu/cell ⇒ 6.18 μl

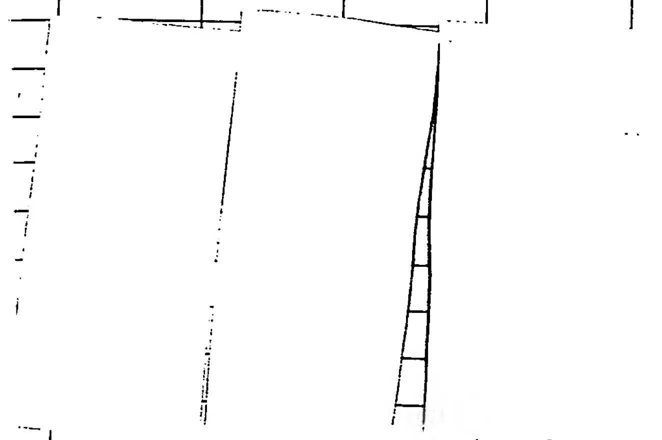
6.12 X 10^9/ml KD1 ⇒ 6.12 x 10^6/μl

50pfu/cell ⇒ 8.16 μl

Harvest 33h p.i.

∅	0.190		
mock	0.280	100	5μl
303	[illegible]	179	2.8μl
KD1	[illegible]	140	3.6μl

4



97
66
45
31
21.5
14.4
6.5

10 and 15% SDS PAGE

[mock, 303, KD1] 3X, BR

transfer / mark 12.5 10%, 4.5 15%

block 10% milk ON

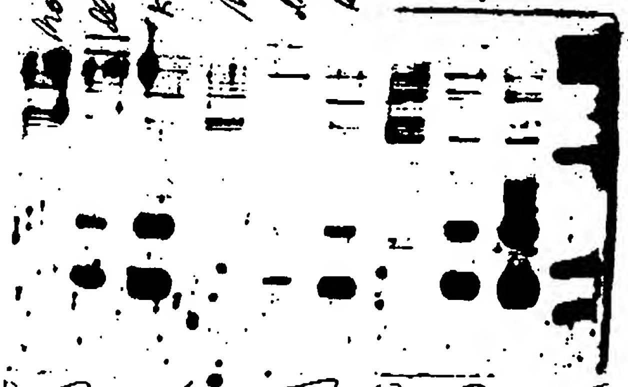
cut	1st ab		wash	2nd ab	wash
4	12522II	1:400	3X	α rabbit HRPO	3X
5	12531				
6	100578				

EXHIBIT

L2

– KD1 expressed Ad5 Ab well
– Can detect Ad5 ADP by Western
– Prelim – looks like KD1 expresses more ADP than 309?
Used 500 PFU of dl309
Used 50 PFU of KD1

mock dl309 KD2 mock dl309 KD1 mock dl309 KD1



APP —
ADP —

αADP Western 3d h pi

Next expt – Time course
KD1
KD2
KD3
1101/1107

3 diff antibodies
12532II 12531 100578
1/100 dilution
Use this

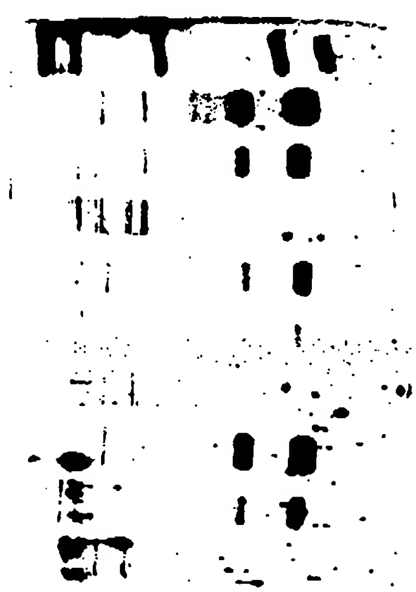


EXHIBIT
L3

12/12/87